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Notes:

1. Untranslatable words are replaced with asterisks (****).
2. Texts in the figures are not translated and shown as it is.

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[Claim(s)]

[Claim 1] The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride is made to act on a specimen under existence of an alternative reaction promoting agent. How to make a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein which perform measurement of the hydrogen peroxide to generate or a returned type coenzyme.

[Claim 2] The method given in the 1st clause of a claim in order that an alternative reaction promoting agent may make a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein which it is.

[Claim 3] The triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent alternatively The method given in the 1st clause of a claim which performs alternatively the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride.

[Claim 4] The method given in the 3rd clause of a claim which is the enzyme with which an alternative reaction promoting agent carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the thing made to react.

[Claim 5] As the 1st step, the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride The triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein is eliminated. as the 2nd step By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride which remains from triglyceride The method given in the 1st clause of a claim which performs alternatively the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein.

[Claim 6] The alternative reaction promoting agent made to exist in the 1st step the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein alternatively The method given in the 5th clause of a claim which is the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and the thing made to react.

[Claim 7] The method given in the 5th clause of a claim an alternative reaction promoting agent which is different from this alternative reaction promoting agent with the alternative reaction promoting agent made to exist in the 1st step in the 2nd step is made to exist.

[Claim 8] [a different alternative reaction promoting agent from the alternative reaction promoting agent made to exist in the 1st step] The method given in the 7th clause of a claim which is the enzyme which carries out the catalyst

of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the thing made to react.

[Claim 9] As the 1st step, the bottom of existence of an alternative reaction promoting agent, very low density lipoprotein, The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein is eliminated. It is (however, not performing elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein). as the 2nd step The triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent among the triglyceride which remains alternatively The method of the claim 1 description which performs alternatively the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride.

[Claim 10] The alternative reaction promoting agent made to exist in the 1st step and the alternative reaction promoting agent made to exist in the 2nd step are the following combination (i) - (iii). : (i)

The 1st step : The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein The 2nd step : In the 1st step The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(ii)

The 1st step : Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in middle specific gravity lipoprotein The 2nd step : In the 1st step The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(iii)

The 1st step : Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which

carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in very low density lipoprotein The 2nd step : In the 1st step The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

The method given in the 9th clause of a claim which it is in *****.

[Claim 11] [the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride] (i) The method given in the 1st clause of a claim which is either of lipoprotein lipase, (ii) glycerol kinase, glycerol 3-phosphorus acid oxidase, and glycerol 3-phosphorus acid DEHIDROGENASE.

[Claim 12] The way given in the 1st clause of a claim which is a thing a specimen may contain at least one sort chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein.

[Claim 13] The way given in the 1st clause of a claim an alternative reaction promoting agent is a surface-active agent, polyoxyalkylene, its derivative, polysaccharide, or its derivative.

[Claim 14] The method given in the 1st clause of a claim a reaction auxiliary substance is made to exist with an alternative reaction promoting agent.

[Claim 15] The way given in the 14th clause of a claim a reaction auxiliary substance is poly ANION, halogen ion, a metal ion, or REKUCHIN.

[Claim 16] (i) The reagent for making a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein containing an alternative reaction promoting agent and the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from (ii) triglyceride in a sample.

[Claim 17] The reagent given in the 16th clause of a claim in order that an alternative reaction promoting agent may make a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein which it is.

[Claim 18] The enzyme with which an alternative reaction promoting agent carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the reagent given in the 16th clause of a claim which is made to react and which is a thing.

[Claim 19] An alternative reaction promoting agent the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein alternatively The reagent given in the 16th clause of a claim which is what eliminates the triglyceride which is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate, and is contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein by this from triglyceride.

[Claim 20] The reagent given in the 16th clause of a claim which is what an alternative reaction promoting agent contains into the 1st reagent by a reagent consisting of the 1st reagent and the 2nd reagent.

[Claim 21] The reagent given in the 16th clause of a claim which is what an alternative reaction promoting agent contains into the 2nd reagent by a reagent consisting of the 1st reagent and the 2nd reagent.

[Claim 22] The reagent given in the 16th clause of a claim which is what an alternative reaction promoting agent contains into the 1st reagent and the 2nd reagent by a reagent consisting of the 1st reagent and the 2nd reagent.

[Claim 23] The reagent given in the 22nd clause of a claim which is an alternative reaction promoting agent which the alternative reaction promoting agent contained into the 2nd reagent is the same as the alternative reaction promoting agent contained into the 1st reagent, or is different.

[Claim 24] [the alternative reaction promoting agent which a reagent contains into the 1st reagent by consisting of the 1st reagent and the 2nd reagent] Make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride react them with the enzyme which carries out a catalyst, and by this It is what eliminates the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein. The alternative reaction promoting agent contained into the 2nd reagent the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein alternatively The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and the reagent given in the 16th clause of a claim which is made to react and which is a thing.

[Claim 25] [the alternative reaction promoting agent which a reagent contains into the 1st reagent by consisting of the 1st reagent and the 2nd reagent] The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively From triglyceride, make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate react them with the enzyme which carries out a catalyst, and by this It is what eliminates said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein. (However, elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein is not performed) The alternative reaction promoting agent contained into the 2nd reagent the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein among the triglyceride which remains alternatively The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and the reagent given in the 16th clause of a claim which is made to react and which is a thing.

[Claim 26] The alternative reaction promoting agent made to contain in the 1st reagent and the alternative reaction promoting agent made to contain in the 2nd reagent are the following combination (i) - (iii). : (i)
The 1st reagent: The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein The 2nd reagent: In the 1st reagent The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(ii)

The 1st reagent: Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in

middle specific gravity lipoprotein The 2nd reagent: In the 1st reagent The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(iii)

The 1st reagent: Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in very low density lipoprotein The 2nd reagent: In the 1st reagent The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

The reagent given in the 25th clause of a claim which it is in *****.

[Claim 27] [the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride] (i) The reagent given in the 16th clause of a claim which is either of lipoprotein lipase, (ii) glycerol kinase, glycerol 3-phosphorus acid oxidase, and glycerol 3-phosphorus acid DEHIDROGENASE.

[Claim 28] The reagent given in the 16th clause of a claim with which a specimen may contain at least one sort chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein and which is a thing.

[Claim 29] The reagent given in the 16th clause of a claim a given alternative reaction promoting agent is a surface-active agent, polyoxyalkylene, its derivative, polysaccharide, or its derivative.

[Claim 30] The reagent given in the 16th clause of a claim a reaction auxiliary substance is made to exist with an alternative reaction promoting agent.

[Claim 31] The reagent given in the 30th clause of a claim a given reaction auxiliary substance is poly ANION, halogen ion, a metal ion, or REKUCHIN.

[Claim 32] In the enzyme colorimetric assay method which carries out a fixed quantity of triglyceride in serum combining lipoprotein lipase, glycerol kinase, glycerol 3-phosphorus acid oxidase (or glycerol 3-phosphorus acid DEHIDROGENASE), etc. How to make a fixed quantity alternatively triglyceride in very low density lipoprotein (for middle specific gravity lipoprotein to also be included in this, since quality is similar) by making positive ion nature, negative ion nature, or a nonionic surface-active agent act.

[Claim 33] In the enzyme measuring method which carries out a fixed quantity of triglyceride in serum combining lipoprotein lipase, glycerol kinase, glycerol 3-phosphorus acid oxidase (or glycerol 3-phosphorus acid DEHIDROGENASE), etc., positive ion nature, negative ion nature, By making a nonionic surface-active agent act, or the Cairo micron, How to make carry out reaction decomposition of the triglyceride in low-density lipoprotein and high density lipoprotein alternatively, and carry out a fixed quantity of triglyceride in very low density lipoprotein (for middle specific gravity lipoprotein to also be included in this, since quality is similar) after that.

[Claim 34] How to add poly ANION, divalent metallic ion, or sugar which promotes the selectivity of this lipoprotein and a surface-active agent in the triglyceride fixed-quantity method in very low density lipoprotein given in the 32nd

clause of a claim.

[Claim 35] How to add poly ANION, divalent metallic ion, or sugar which promotes the selectivity of the Cairo micron, and a LDL and HDL and a surface-active agent in the triglyceride fixed-quantity method in very low density lipoprotein given in the 33rd clause of a claim.

[Detailed Description of the Invention]

Technical field This invention relates to the alternative fixed-quantity method of triglyceride and alternative fixed-quantity reagent which are contained in the very low density lipoprotein important for a clinic test and/or middle specific gravity lipoprotein of arteriosclerosis.

Background art Cholesterol and triglyceride are nutrients indispensable for a living body. Since these do not melt into water easily, they are wrapped in an amphiphilic film and exist in blood (as lipoprotein).

In lipoprotein, the Cairo micron, very low density lipoprotein (Very Low Density Lipoprotein;VLDL), Middle specific gravity lipoprotein (Intermediate Density Lipoprotein;IDL), There is a kind of low-density lipoprotein (Low Density Lipoprotein;LDL), high density lipoprotein (High Density Lipoprotein;HDL), etc., and the complicated metabolism system is formed.

Although each lipoprotein contains cholesterol and triglyceride, about VLDL and IDL, triglyceride is the main ingredients and it is deeply concerned with generating of arteriosclerosis. Therefore, it is useful to carry out a judgment fixed quantity of the triglyceride of VLDL and IDL.

According to some large-scale follow-up surveys which investigated many factors which participate in generating of arteriosclerosis, it is proved precipitative [LDL cholesterol and the total amount (it is hereafter called the total triglyceride) of triglyceride in serum] that HDL cholesterol acts restrainedly.

There is almost no triglyceride in LDL and HDL, and most is contained in the Cairo micron, VLDL, and IDL.

On the other hand, it is shown that triglyceride in the Cairo micron is not the dangerous factor of arteriosclerosis. Therefore, only the Cairo micron is eliminated, and the temporary purpose is reached even if it carries out a fixed quantity of triglyceride in other lipoprotein.

The method of carrying out a fixed quantity of the total triglyceride, without classifying various lipoprotein already exists. It is used widely (Henry, J.B., Clinical Diagnosis and Management by Laboratory Method, Philadelphia:W.B. Saunders, pp.196-198).

These methods decompose triglyceride in serum into a GURISE roll with lipoprotein lipase first. Next, this is changed to glycerol 3-phosphorus acid by glycerol kinase, it changes into dihydroxy acetone 3-phosphorus acid with glycerol 3-phosphorus acid oxidase further, and a coloring fixed quantity of the hydrogen peroxide then generated is carried out by a peroxidase system.

Moreover, glycerol 3-phosphorus acid DEHYDROGENASE is made to act instead of glycerol 3-phosphorus acid oxidase, and there is also the method of carrying out a fixed quantity of generated NADH(s). These are widely called the enzyme measuring method.

On the other hand, a specific surface-active agent and a specific additive agent are made to act on LDL or HDL alternatively, how to carry out a fixed quantity of cholesterol contained there is also already known (for example, JP, H9-313200,A and JP,H9-285298,A), and it is widely used for the purpose, such as a clinical examination.

Whether it acts on VLDL and IDL alternatively However, or the Cairo micron, The method and reagent which make a fixed quantity alternatively the triglyceride which the combination of a reagent which eliminates LDLHDL is not known yet, therefore is contained in front 2 persons' lipoprotein (VLDL, IDL) are not indicated until now.

Indication of invention The technical problem which it is going to solve by this invention is establishment of a method and a reagent which makes a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein in a sample.

Complicated operation of the separation operation by a ultra-centrifugal separation machine etc. is not more specifically needed. It is establishing the method and reagent which make a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein which application

to the automatic analysis equipment currently used widely is possible, and can perform a fixed quantity simple and correctly.

This invention includes the following invention.

- (1) Make the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride act on a specimen under existence of an alternative reaction promoting agent. How to make a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein which perform measurement of the hydrogen peroxide to generate or a returned type coenzyme.
- (2) A method given in the above (1) which it is in order that an alternative reaction promoting agent may make a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein.
- (3) The triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent alternatively A method the above (1) which performs alternatively the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, or given in (2).
- (4) A method given in the enzyme with which an alternative reaction promoting agent carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the above (3) which is the thing made to react.
- (5) As the 1st step, the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride The triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein is eliminated. as the 2nd step By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride which remains from triglyceride A method the above (1) which performs alternatively the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein, or given in (2).
- (6) The alternative reaction promoting agent made to exist in the 1st step the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein alternatively A method given in the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and the above (5) which is the thing made to react.
- (7) A method the above (5) in which an alternative reaction promoting agent which is different from this alternative reaction promoting agent with the alternative reaction promoting agent made to exist in the 1st step in the 2nd step is made to exist, or given in (6).
- (8) [a different alternative reaction promoting agent from the alternative reaction promoting agent made to exist in the 1st step] A method given in the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the above (7) which is the thing made to react.

As the 1st step, (9) The bottom of existence of an alternative reaction promoting agent, very low density lipoprotein, The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein is eliminated. It is (however, not performing elimination of both

triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein). as the 2nd step The triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent among the triglyceride which remains alternatively A method the above (1) which performs alternatively the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, or given in (2).

(10) The alternative reaction promoting agent made to exist in the 1st step and the alternative reaction promoting agent made to exist in the 2nd step are the following combination (i) - (iii). : (i)

The 1st step : The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein The 2nd step : In the 1st step The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(ii)

The 1st step : Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in middle specific gravity lipoprotein The 2nd step : In the 1st step The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(iii)

The 1st step : Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in very low density lipoprotein The 2nd step : In the 1st step The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the

alternative reaction promoting agent which can be made to react;

A method given in the above (9) which it is in *****.

(11) [the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride] (i) A method given in either of aforementioned (1) - (10) which is either of lipoprotein lipase, (ii) glycerol kinase, glycerol 3-phosphorus acid oxidase, and glycerol 3-phosphorus acid DEHIDROGENASE.

(12) A method given in either of aforementioned (1) - (11) which is that in which a specimen may contain at least one sort chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein.

(13) A method given in either of aforementioned (1) - (12) whose alternative reaction promoting agent is a surface-active agent, polyoxyalkylene, its derivative, polysaccharide, or its derivative.

(14) A method given in either of aforementioned (1) - (13) in which a reaction auxiliary substance is made to exist with an alternative reaction promoting agent.

(15) A method given in the above (14) whose reaction auxiliary substance is poly ANION, halogen ion, a metal ion, or REKUCHIN.

(16) Reagent for making a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein containing (i) alternative reaction promoting agent and the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from (ii) triglyceride in a sample.

(17) A reagent given in the above (16) which it is in order that an alternative reaction promoting agent may make a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein.

(18) An alternative reaction promoting agent the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein alternatively A reagent the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, the above (16) which is the thing made to react, or given in (17).

(19) An alternative reaction promoting agent the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein alternatively A reagent the above (16) which is what eliminates the triglyceride which is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate, and is contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein by this from triglyceride, or given in (17).

(20) A reagent given in either of aforementioned (16) - (19) which is what an alternative reaction promoting agent contains into the 1st reagent by a reagent consisting of the 1st reagent and the 2nd reagent.

(21) A reagent given in either of aforementioned (16) - (19) which is what an alternative reaction promoting agent contains into the 2nd reagent by a reagent consisting of the 1st reagent and the 2nd reagent.

(22) A reagent given in either of aforementioned (16) - (19) which is what an alternative reaction promoting agent contains into the 1st reagent and the 2nd reagent by a reagent consisting of the 1st reagent and the 2nd reagent.

(23) A reagent given in the above (22) which is an alternative reaction promoting agent which the alternative reaction promoting agent contained into the 2nd reagent is the same as the alternative reaction promoting agent contained into the 1st reagent, or is different.

(24) [the alternative reaction promoting agent which a reagent contains into the 1st reagent by consisting of the 1st reagent and the 2nd reagent] Make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride react them with the enzyme which carries out a catalyst, and by this It is what eliminates the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein. The alternative reaction promoting agent contained into the 2nd reagent the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein alternatively A reagent the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type

coenzyme generate from triglyceride, the above (16) which is the thing made to react, or given in (17).

(25) [the alternative reaction promoting agent which a reagent contains into the 1st reagent by consisting of the 1st reagent and the 2nd reagent] The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively From triglyceride, make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate react them with the enzyme which carries out a catalyst, and by this It is what eliminates said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein. (However, elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein is not performed) The alternative reaction promoting agent contained into the 2nd reagent the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein among the triglyceride which remains alternatively A reagent the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, the above (16) which is the thing made to react, or given in (17).

(26) The alternative reaction promoting agent made to contain in the 1st reagent and the alternative reaction promoting agent made to contain in the 2nd reagent are the following combination (i) - (iii). : (i)

The 1st reagent: The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein The 2nd reagent: In the 1st reagent The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(ii)

The 1st reagent: Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in middle specific gravity lipoprotein The 2nd reagent: In the 1st reagent The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

(iii)

The 1st reagent: Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density

lipoprotein, And alternative reaction promoting agent which can eliminate said triglyceride contained in very low density lipoprotein The 2nd reagent: In the 1st reagent The Cairo micron which reacted alternatively with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react;

A reagent given in the above (25) which it is in *****.

(27) [the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride] (i) A reagent given in either of aforementioned (16) - (26) which is either of lipoprotein lipase, (ii) glycerol kinase, glycerol 3-phosphorus acid oxidase, and glycerol 3-phosphorus acid DEHIDROGENASE.

(28) A reagent given in either of aforementioned (16) - (27) which is that in which a specimen may contain at least one sort chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein.

(29) A reagent given in either of aforementioned (16) - (28) whose alternative reaction promoting agent is a surface-active agent, polyoxyalkylene, its derivative, polysaccharide, or its derivative.

(30) A reagent given in either of aforementioned (16) - (29) in which a reaction auxiliary substance is made to exist with an alternative reaction promoting agent.

(31) A reagent given in the above (30) whose reaction auxiliary substance is poly ANION, halogen ion, a metal ion, or REKUCHIN.

In the enzyme colorimetric assay method which carries out a fixed quantity of triglyceride in serum combining lipoprotein lipase, glycerol kinase, glycerol 3-phosphorus acid oxidase (or glycerol 3-phosphorus acid DEHIDROGENASE), etc., (32) Positive ion nature, negative ion nature, Or the method of making a fixed quantity alternatively triglyceride in very low density lipoprotein (middle specific gravity lipoprotein also being included in this, since quality is similar) by making a nonionic surface-active agent act.

In the enzyme measuring method which carries out a fixed quantity of triglyceride in serum combining lipoprotein lipase, glycerol kinase, glycerol 3-phosphorus acid oxidase (or glycerol 3-phosphorus acid DEHIDROGENASE), etc., (33) Positive ion nature, negative ion nature, By making a nonionic surface-active agent act, or the Cairo micron, How to make carry out reaction decomposition of the triglyceride in low-density lipoprotein and high density lipoprotein alternatively, and carry out a fixed quantity of triglyceride in very low density lipoprotein (for middle specific gravity lipoprotein to also be included in this, since quality is similar) after that.

(34) How to add poly ANION, divalent metallic ion, or sugar which promotes the selectivity of this lipoprotein and a surface-active agent in the triglyceride fixed-quantity method in very low density lipoprotein given in the above (32).

(35) How to add poly ANION, divalent metallic ion, or sugar which promotes the selectivity of the Cairo micron, and a LDL and HDL and a surface-active agent in the triglyceride fixed-quantity method in very low density lipoprotein given in the above (33).

This invention is explained in detail hereafter.

I. Method and introduction which is made fixed quantity and which method 1. fixed quantity carries out [the method of making a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein of this invention] It becomes a specimen under existence of an alternative reaction promoting agent from performing measurement of the hydrogen peroxide which the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate is made to act, and is generated from triglyceride, or a returned type coenzyme.

In the method of this this invention, an alternative reaction promoting agent is for making a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein.

2. Mode ** Mode-1 of Fixed Quantity of Methods [The 1st Method of this Invention]

In the method of said this invention, one of the modes of the method of this invention Under existence of an alternative reaction promoting agent, By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride The fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein is performed alternatively. (The 1st method of this invention)

Under the present circumstances, an alternative reaction promoting agent is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride.

In addition, when dividing a fixed quantity into two steps, the 1st step and the 2nd step, and performing it, an alternative reaction promoting agent may be made to exist in the 1st step, and may be made to exist in the 2nd step, or may be made to exist in the 1st step and the 2nd step. The effect same in any case can be acquired.

Moreover, it can also be used combining two or more kinds of alternative reaction promoting agents, making it exist simultaneously.

** Mode-2 [The 2nd method of this invention]

Moreover, the following methods can be mentioned as another thing of the mode of the method of this invention.

In the method of said this invention, as the 1st step, first Under existence of an alternative reaction promoting agent, By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride The triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein is eliminated (decomposition).

Next, by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride which remains from triglyceride as the 2nd step The fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein is performed alternatively. (The 2nd method of this invention)

[under the present circumstances, the alternative reaction promoting agent made to exist in the 1st step] It is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride.

In addition, you may make an alternative reaction promoting agent which is different from this alternative reaction promoting agent with the alternative reaction promoting agent made to exist in the 1st step exist in the 2nd step.

[in this case, a different alternative reaction promoting agent from the alternative reaction promoting agent made to exist in the 1st step] It is desirable that they are the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the thing made to react.

** Mode-3 [The 3rd method of this invention]

Furthermore, the following methods can be mentioned as other things of the mode of the method of this invention.

In the method of said this invention, as the 1st step, first Under existence of an alternative reaction promoting agent, The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein is eliminated (decomposition). (However, elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are

contained in very low density lipoprotein is not performed.)

Next, the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent as the 2nd step among the triglyceride which remains alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein is performed alternatively. (The 3rd method of this invention)

Under the present circumstances, the alternative reaction promoting agent made to exist in the 1st step and the alternative reaction promoting agent made to exist in the 2nd step can also be chosen from following (i) - the combination of (iii).

(i)

The 1st step : The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride The alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein.

The 2nd step : The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the 1st step, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react.

(ii)

The 1st step : Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride The alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, and said triglyceride contained in middle specific gravity lipoprotein.

The 2nd step : The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the 1st step, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react.

(iii)

The 1st step : Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride The alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, and said triglyceride contained in very low density lipoprotein.

The 2nd step : The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the 1st step, and the Cairo micron which reacted alternatively,

The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react.

3. A Series of Reactions Which Make Hydrogen Peroxide or Returned Type Coenzyme Generate from Enzyme ** Triglyceride Which Carries Out Catalyst of a Series of Reactions Which Make Hydrogen Peroxide or Returned Type Coenzyme Generate from Triglyceride It Can Set to the Method of this Invention. [a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride] As long as it is the reaction which can make hydrogen peroxide or a returned type coenzyme generate when triglyceride exists, what kind of thing may be used, and it may consist of one reaction and you may consist of two or more reactions.

For example, lipoprotein lipase is made to act on triglyceride of the lipoprotein in a sample as such a reaction. This triglyceride is decomposed into the GURISE roll of one molecule, and fatty acid of three molecules. This GURISE roll and adenosine triphosphate (ATP) are changed into glycerol 3-phosphorus acid and adenosine diphosphoric acid (ADP) by the catalytic action of glycerol kinase after that. Furthermore, while changing this glycerol 3-phosphorus acid into dihydroxy acetone 3-phosphorus acid by the catalytic action of glycerol 3-phosphorus acid oxidase, a series of reactions which produce hydrogen peroxide can be mentioned.

Moreover, lipoprotein lipase is made to act on triglyceride of the lipoprotein in a sample as other examples. This triglyceride is decomposed into the GURISE roll of one molecule, and fatty acid of three molecules. This GURISE roll and adenosine triphosphate (ATP) are changed into glycerol 3-phosphorus acid and adenosine diphosphoric acid (ADP) by the catalytic action of glycerol kinase after that. This glycerol 3-phosphorus acid Furthermore, under existence of nicotine amide adenine dinucleotide (oxidized type) [NAD⁺], While changing into dihydroxyacetone phosphate by the catalytic action of glycerol 3-phosphorus acid DEHIDROGENASE, a series of reactions which produce nicotine amide adenine dinucleotide (returned type) [NADH] can be mentioned. In addition, it sets for the reaction using the aforementioned glycerol kinase. Since a positive error may arise in a fixed-quantity value if a GURISE roll is included in a sample In order to prevent this, you may make glycerol kinase and glycerol 3-phosphorus acid oxidase, and a series of reactions that KATARAZE or peroxidase is made to act further and eliminate this GURISE roll perform on the GURISE roll included in a sample beforehand.

When using KATARAZE and performing the fixed quantity of triglyceride after the end of an elimination reaction, the substance which checks the activity of KATARAZE, such as sodium azide, is made to exist, and the generated hydrogen peroxide needs to be made not to be eliminated here by KATARAZE (decomposition).

In addition, as a returned type coenzyme, nicotine amide adenine dinucleotide (returned type) [NADH (returned type)] or nicotinamide adenine dinucleotide phosphate (returned type) [NADPH (returned type)] can be mentioned.

** Enzyme [the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the method of this invention] [as long as it carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, what kind of thing may be used, but] For example, lipoprotein lipase and glycerol kinase and also glycerol 3-phosphorus acid oxidase, or glycerol 3-phosphorus acid DEHIDROGENASE etc. can be mentioned.

The thing of animal origin, such as a thing of microbe origin, such as bacteria or mold, humans, or a cow, the thing of vegetable origin, or the thing prepared by the modifying-gene method can be used for these enzyme, for example.

What is necessary is just to make it exist generally by ***** and the concentration which was suitable for the condition suitably, although it is the concentration in which these enzyme is made to exist, since it changes with the kind of the kind of enzyme and the origin, and alternative reaction promoting agent, or mixed ratios of the 1st reagent and the 2nd reagent.

In addition, as for lipoprotein lipase, it is desirable to make it exist by the concentration of 1 - 10,000,000 unit / l, and especially its thing made to exist by the concentration of 100 - 1,000,000 unit / l is usually desirable.

Moreover, as for glycerol kinase, it is usually desirable to make it exist by the concentration of 0.01 - 500,000 unit / l,

and especially its thing made to exist by the concentration of 10 - 10,000 unit / l is desirable.

And as for glycerol 3-phosphorus acid oxidase, it is usually desirable to make it exist by the concentration of 1 - 500,000 unit / l, and especially its thing made to exist by the concentration of 100 - 50,000 unit / l is desirable.

In addition, originally, the activity value of enzyme changes with activity measurement methods, and even if they are the same activity measurement method and the same enzyme, it also changes with the origins or the degrees of refining of the enzyme etc.

Therefore, it is not that the enzyme concentration (enzyme activity value) which separates from the concentration range of each enzyme indicated previously, even so the effect of this invention are not acquired.

**** Substances other than enzyme** It will be made to exist if there is a substance required besides the aforementioned enzyme in a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the method of this invention.

As a substance required for this reaction, adenosine triphosphate (ATP) or its salt, magnesium ion, or an oxidized type coenzyme can be mentioned, for example.

As for the concentration in which adenosine triphosphate or its salt is made to exist, 0.001-50g/l is desirable, and especially its 0.01-10g/l is usually desirable.

As for magnesium ion, it is [that what is necessary is just to use the thing of the form of salt with halogen ion or organic acid] usually desirable to make it exist by the concentration of 0.001 - 100mM; and especially its thing made to exist by the concentration of 0.01 - 50mM is desirable.

As an oxidized type coenzyme, nicotine amide adenine dinucleotide (oxidized type) [NAD⁺ (oxidized type)] or nicotinamide adenine dinucleotide phosphate (oxidized type) [NADP⁺ (oxidized type)] can be mentioned, for example.

4. Measurement of hydrogen peroxide or returned type coenzyme As long as measurement of the hydrogen peroxide to generate or the returned type coenzyme in how to make this invention a fixed quantity is the method of measuring the quantity of the hydrogen peroxide generated with the aforementioned enzyme, or a returned type coenzyme, it may be what kind of method.

for example, what from the generated hydrogen peroxide or a returned type coenzyme -- it is -- the method of drawing a signal etc. can be mentioned.

In measurement of hydrogen peroxide, hydrogen peroxide itself may be measured with a hydrogen peroxide electrode etc., or another signal may be drawn from hydrogen peroxide and, more specifically, this signal may be measured.

The reaction using the Trinder system of reaction which oxidize a **** object on the basis of the existence of peroxidase (POD), it is made to generate a pigment as a method of drawing and measuring another signal from this hydrogen peroxide for example, and measures ***** of this generated pigment etc. can be mentioned.

The thing of vegetable origin; such as a thing of animal origin, such as a thing of microbe origin, humans, or a cow, horseradishes, etc., such as bacteria or mold, or the thing prepared by the modifying-gene method can be used for peroxidase, for example.

As for the concentration in which this peroxidase is made to exist, it is usually desirable to carry out to more than 30 units / l.

As a **** object in the Trinder system of reaction, the combination of 4-amino anti PIRIN, phenol, its derivative, or a 4-amino anti PIRIN and an aniline derivative etc. can be mentioned, for example.

As for 4-amino anti PIRIN, it is usually desirable to make it exist by the concentration of 0.001-50g/l, and especially its thing made to exist by the concentration which is 0.01-10g/l is desirable.

As a derivative of phenol, 4-chloro phenol, 2, 4-dichloro phenol, 2, 4-dibromophenol or 2 and 4, 6-bird chloro phenol, or these salt can be mentioned, for example.

As an aniline derivative, for example N-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy aniline (HDAOS), N-sulfopropyl 3, 5-dimethoxy aniline (HDAPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy aniline (DAOS), N-ethyl N-sulfopropyl 3, 5-dimethoxy aniline (DAPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy 4-fluoro aniline (FDAOS), N-ethyl N-sulfopropyl 3, 5-dimethoxy 4-fluoro aniline (FDAPS), N-(2-carboxyethyl)-N-ethyl 3, 5-

dimethoxy aniline (CEDB), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3-methoxyaniline (ADOS), N-ethyl N-(3-sulfopropyl)-3-methoxyaniline (ADPS), N-ethyl N-(2-hydroxy 3-sulfopropyl) aniline (ALOS), N-ethyl N-(3-sulfopropyl) aniline (ALPS), N-(3-sulfopropyl) aniline (HALPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3, 5-JIMECHIRU aniline (MAOS), N-ethyl N-(3-sulfopropyl)-3, 5-JIMECHIRU aniline (MAPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3-methoxyaniline (TOOS), N-(2-carboxyethyl)-N-ethyl 3-methylaniline (CEMB), N-(2-carboxyethyl)-N-ethyl 3-methoxyaniline (CEMO), or these salt can be mentioned.

As for these phenol, the derivative of those, or an aniline derivative, it is usually desirable to make it exist by the concentration of 0.001-50g/l, and especially its thing made to exist by the concentration which is 0.01-10g/l is desirable.

Moreover, in measurement of a returned type coenzyme, you may measure by measuring ***** [in / in the returned type coenzyme itself / 340 etc.nm etc.] etc., or another signal may be drawn from a returned type coenzyme, and this signal may be measured.

For example, tetrazolium salt etc. can be made to be able to return to the basis of existence, such as JIAHORAZE or 1-*****_***** sulfate, a pigment can be made to be able to generate as a method of drawn and measuring another signal from this returned type coenzyme, and the reaction which measures this can be mentioned.

5. Elimination Reaction In the 2nd Method of this Invention In the 1st step, the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein under existence of an alternative reaction promoting agent alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein is eliminated (decomposition).

In the 3rd method of this invention, are the 1st step and Moreover, under existence of an alternative reaction promoting agent, The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein is eliminated (decomposition). (However, elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein is not performed) .

Although these elimination (decomposition) is performed by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate a sample from triglyceride under existence of an alternative reaction promoting agent The details about a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from this triglyceride are as having already described.

As for the hydrogen peroxide or the returned type coenzyme generated by the reaction of this elimination (decomposition), it is desirable to eliminate in the 1st step, without carrying over to the 2nd step.

In the case of hydrogen peroxide, it is eliminable by making KATARAZE or peroxidase exist, for example (decomposition).

The thing of animal origin, such as a thing of microbe origin, such as bacteria or mold, humans, or a cow, the thing of vegetable origin, or the thing prepared by the modifying-gene method can be used for KATARAZE, for example.

As for the concentration in which this KATARAZE is made to exist, it is usually desirable to carry out to more than 100 units / l.

And after eliminating the hydrogen peroxide generated by KATARAZE in the 1st step, the activity of KATARAZE is checked in the 2nd step and it is necessary to make it not work.

This can be attained by making the substance which checks the activity of KATARAZE, such as sodium azide, exist in the 2nd step.

Moreover, the thing of vegetable origin, such as a thing of animal origin, such as a thing of microbe origin, humans, or a cow, horseradishes, etc., such as bacteria or mold, or the thing prepared by the modifying-gene method can be

used for peroxidase, for example.

As for the concentration in which this peroxidase is made to exist, it is usually desirable to carry out to more than 30 units / l.

In the case of a returned type coenzyme, it is eliminable by, for example, making the dehydration enzyme which uses this returned type coenzyme as a coenzyme exist (decomposition).

6. Other Substances In How to Make this Invention Fixed Quantity Further if needed The ground substance of a buffer, other enzyme, and other enzyme, other coenzymes, The salt containing metal ions, such as alkaline metal salt or alkaline-earth-metals salt, or this, Protein, such as a chelating agent and albumin, sodium azide, an antibiotic, Or a substance in connection with elimination or influence control, a diluent base, or other reagent ingredients of the measurement interfering substance contained in samples, such as stabilizers, such as antiseptics, such as synthetic antibacteria medicine, sugars, or a high molecular compound, an activating agent, and ascorbate oxidase, etc. can be made to exist if needed suitably.

In the reagent of this invention, as for pH at the time of mixing a sample and a reagent and performing a fixed quantity, it is desirable that it is pH five to 10 range, and it is desirable that it is especially pH 5.5 to 9.0 range. When performing a fixed quantity from the 1st step and the 2nd step, you may set up pH of the 1st step so that pH of the 2nd step may serve as the range of the aforementioned pH.

When making a buffer exist, it is desirable to make a buffer which serves as the range of pH of the above [pH at the time of performing a fixed quantity] exist.

For example, MES, Bis-Tris, Bis-Tris propane, ADA, PIPES, ACES, MOPSO, MOPS, BES, TES, HEPES, DIPSO, TAPSO, POPSO, HEPES, HEPPSO, EPPS, Tricine, Bicine, TAPS, CHES, phosphorus acid, an orthophosphate, boric acid, borate salt, Grishin, GURISHIRU Grishin, IMIDAZORU, or tris (hydroxymethyl) amino methane [Tris] can be mentioned.

5. Procedure of Method Made Fixed Quantity [How to Make this Invention Fixed Quantity] [the sample which tries to perform the fixed quantity of the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein] The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride is made to act under existence of an alternative reaction promoting agent, and measurement of the hydrogen peroxide to generate or a returned type coenzyme is performed.

You may carry out fixed-quantity operation of the method of this this invention by the method (the multi-step method, multi-reagent system) which may enforce by the method (the 1 step method, 1 reagent system) of performing only in one step, or is performed by two or more steps, such as two etc. steps.

Which methods, such as the method of performing by adding a fixed quantity of ground substances or substances indispensable for a fixed-quantity reaction or the method of performing by adding a sample, are sufficient as the start method of a reaction.

The temperature at the time of operation should just set up the temperature within the limits in which fixed-quantity reactions, such as 30 degrees C or 37 etc. degrees C, advance, and they do not deteriorate [reaction ingredients, such as enzyme concerning a fixed-quantity reaction, are not deactivated, or] with heat in fixed quantity.

Moreover, in the method of this invention, measurement of the hydrogen peroxide to generate or a returned type coenzyme may be based on which methods, such as a reaction velocity method (the rate method) or a terminal point method (the endpoint method).

In measurement of the hydrogen peroxide to generate or a returned type coenzyme, when measurement of ***** etc. performs this, the measurement wavelength should just use the suitable wavelength of the purple exterior, a visible portion, or the red exterior according to the substance to measure.

In addition, one wave of measurement is sufficient as measurement of ***** etc., or measurement by two waves is sufficient as it.

in the method of this invention -- a fixed quantity of techniques -- business -- all of the method of depending on equipment, such as a technique or automatic analysis equipment, can be used.

As this equipment, the automatic analysis equipment for clinical examinations etc. can be mentioned, for example.

As an example of the automatic analysis equipment for these clinical examinations, the automatic analysis equipment of flow methods, such as a continuous flow type or a flow injection type, The automatic analysis equipment of dry chemistry methods, such as automatic analysis equipment of discrete methods, such as a closed type batch type, an open type batch type, a pack type, or a centrifugal type, a film type, or a specimen type, etc. can be mentioned. An example of a procedure which performs a fixed quantity with equipment is shown below.

First, the reagent which this invention makes a fixed quantity is put into the container which suited the equipment to be used.

The container containing this reagent is put on the predetermined position of equipment.

Moreover, the sample which performs a fixed quantity is also paid to the container which suited the measuring device, and is put on a predetermined position.

When equipment is automatic analysis equipment for clinical examinations, a fixed quantity of conditions (fixed-quantity parameter) etc. are inputted into equipment about the reagent to be used, the sample which tries to perform a fixed quantity, etc., and it sets up.

And a fixed quantity is started.

Usually, by the pipette (probe) or a tube, it mixes, each of a sample and a reagent is poured distributively and contacted in a reaction cell (reaction KYUBETTO), and it maintains under the condition of temperature regularity.

And it measures at the time which was able to define ***** of the regulation wavelength about the reaction liquid of the sample in this reaction cell (reaction KYUBETTO), and a reagent.

Moreover, for example, when a reagent consists of 2 of the 1st reagent and the 2nd reagent reagents, by the pipette (probe) or a tube, it mixes, each of the 1st reagent of a sample and a reagent is poured distributively and contacted in a reaction cell (reaction KYUBETTO), and it maintains under the condition of temperature regularity first.

next -- pouring the 2nd reagent of a reagent distributively by the pipette (probe) or a tube, mixing and making the reaction liquid in this reaction cell (reaction KYUBETTO) contact -- temperature -- it maintains under a certain condition.

And it measures at the time which was able to define ***** of the regulation wavelength about reaction liquid with the sample in this reaction cell (reaction KYUBETTO), the 1st reagent of a reagent, and the 2nd reagent.

By measuring ***** obtained here and ***** [analytical curve] of the triglyceride sample (reference solution) of concentration known, the concentration of the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein in a sample is computed and obtained.

II. reagent 1. made a fixed quantity -- the reagent and introduction made a fixed quantity [the reagent for making a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein in the sample of this invention] (i) Contain an alternative reaction promoting agent and the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from (ii) triglyceride.

In the reagent of this this invention, an alternative reaction promoting agent is for making a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein.

2. Mode ** Mode-1 of Reagent [1st Reagent of this Invention]

[one of the modes of the reagent of this invention / the alternative reaction promoting agent made to contain in the reagent of said this invention] It is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride. (The 1st reagent of this invention)

In addition, when a reagent consists of the 1st reagent and the 2nd reagent, the 1st reagent may be made to contain an alternative reaction promoting agent, and the 2nd reagent may be made to contain it, or the 1st reagent and the 2nd reagent may be made to contain it. The effect same in any case can be acquired.

Moreover, it can also be used combining two or more kinds of alternative reaction promoting agents, making it contain simultaneously.

** Mode-2 [The 2nd reagent of this invention]

Moreover, the following reagents can be mentioned as another thing of the mode of the reagent of this invention. A reagent consists of the 1st reagent and the 2nd reagent in the reagent of said this invention. The alternative reaction promoting agent contained into the 1st reagent the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein alternatively It is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and this eliminates the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein (decomposition). (The 2nd reagent of this invention)

In addition, you may make an alternative reaction promoting agent which is different from this alternative reaction promoting agent with the alternative reaction promoting agent which the 1st reagent was made to contain contain in the 2nd reagent.

[in this case, a different alternative reaction promoting agent from the alternative reaction promoting agent the 2nd reagent is made to contain and which the 1st reagent was made to contain] It is desirable that they are the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the thing made to react.

** Mode-3 [The 3rd reagent of this invention]

Furthermore, the following reagents can be mentioned as other things of the mode of the reagent of this invention. A reagent consists of the 1st reagent and the 2nd reagent in the reagent of said this invention. The alternative reaction promoting agent contained into the 1st reagent Very low density lipoprotein, middle specific gravity lipoprotein, The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively From triglyceride, make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate react them with the enzyme which carries out a catalyst, and by this Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein is eliminated (decomposition). (However, elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein is not performed.)

And the alternative reaction promoting agent contained into the 2nd reagent the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein among the triglyceride which remains alternatively It is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride. (The 3rd reagent of this invention)

Under the present circumstances, the alternative reaction promoting agent made to contain in the 1st reagent and the alternative reaction promoting agent made to contain in the 2nd reagent can also be chosen from following (i) - the combination of (iii).

(i)

The 1st reagent: The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively By making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride The alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein.

The 2nd reagent: The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the 1st reagent, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react.

(ii)

The 1st reagent: Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the

Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride The alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, and said triglyceride contained in middle specific gravity lipoprotein.

The 2nd reagent: The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the 1st reagent, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react.

(iii)

The 1st reagent: Triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride The alternative reaction promoting agent which can eliminate said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, and said triglyceride contained in very low density lipoprotein.

The 2nd reagent: The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the 1st reagent, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the alternative reaction promoting agent which can be made to react.

3. A Series of Reactions Which Make Hydrogen Peroxide or Returned Type Coenzyme Generate from Enzyme **
Triglyceride Which Carries Out Catalyst of a Series of Reactions Which Make Hydrogen Peroxide or Returned Type Coenzyme Generate from Triglyceride It Can Set into Reagent of this Invention. [a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride] As long as it is the reaction which can make hydrogen peroxide or a returned type coenzyme generate when triglyceride exists, what kind of thing may be used, and it may consist of one reaction and you may consist of two or more reactions.

For example, lipoprotein lipase is made to act on triglyceride of the lipoprotein in a sample as such a reaction. This triglyceride is decomposed into the GURISE roll of one molecule, and fatty acid of three molecules. This GURISE roll and adenosine triphosphate (ATP) are changed into glycerol 3-phosphorus acid and adenosine diphosphoric acid (ADP) by the catalytic action of glycerol kinase after that. Furthermore, while changing this glycerol 3-phosphorus acid into dihydroxy acetone 3-phosphorus acid by the catalytic action of glycerol 3-phosphorus acid oxidase, a series of reactions which produce hydrogen peroxide can be mentioned.

Moreover, lipoprotein lipase is made to act on triglyceride of the lipoprotein in a sample as other examples. This triglyceride is decomposed into the GURISE roll of one molecule, and fatty acid of three molecules. This GURISE roll and adenosine triphosphate (ATP) are changed into glycerol 3-phosphorus acid and adenosine diphosphoric acid (ADP) by the catalytic action of glycerol kinase after that. This glycerol 3-phosphorus acid Furthermore, under existence of nicotine amide adenine dinucleotide (oxidized type) [NAD⁺], While changing into dihydroxy acetone 3-phosphorus acid by the catalytic action of glycerol 3-phosphorus acid DEHIDROGENAZE, a series of reactions which produce nicotine amide adenine dinucleotide (returned type) [NADH] can be mentioned.

In addition, it sets for the reaction using the aforementioned glycerol kinase. Since a positive error may arise in a

fixed-quantity value if a GURISE roll is included in a sample. In order to prevent this, you may make glycerol kinase and glycerol 3-phosphorus acid oxidase, and a series of reactions that KATARAZE or ** is made to act further and eliminate this GURISE roll perform on the GURISE roll included in a sample beforehand.

When using KATARAZE and performing the fixed quantity of triglyceride after the end of an elimination reaction, the 2nd reagent is made to contain the substance which checks the activity of KATARAZE, such as sodium azide, and the generated hydrogen peroxide needs to be made not to be eliminated here by KATARAZE (decomposition). In addition, as a returned type coenzyme, nicotine amide adenine dinucleotide (returned type) [NADH (returned type)] or nicotinamide adenine dinucleotide phosphate (returned type) [NADPH (returned type)] can be mentioned.

** Enzyme [the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in the reagent of this invention] [as long as it carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, what kind of thing may be used, but] For example, lipoprotein lipase and glycerol kinase and also glycerol 3-phosphorus acid oxidase, or glycerol 3-phosphorus acid DEHIDROGENAZE etc. can be mentioned.

The thing of animal origin, such as a thing of microbe origin, such as bacteria or mold, humans, or a cow, the thing of vegetable origin, or the thing prepared by the modifying-gene method can be used for these enzyme, for example. What is necessary is just to make it contain generally by ***** and the concentration which was suitable for the condition suitably, although it is the concentration which makes a reagent contain these enzyme, since it changes with the kind of the kind of enzyme and the origin, and alternative reaction promoting agent, or mixed ratios of the 1st reagent and the 2nd reagent.

In addition, as for lipoprotein lipase, it is desirable to make it contain by the concentration of 1 - 10,000,000 unit / l, and especially its thing made to contain by the concentration of 100 - 1,000,000 unit / l is usually desirable.

Moreover, as for glycerol kinase, it is usually desirable to make it contain by the concentration of 0.01 - 500,000 unit / l, and especially its thing made to contain by the concentration of 10 - 10,000 unit / l is desirable.

And as for glycerol 3-phosphorus acid oxidase, it is usually desirable to make it contain by the concentration of 1 - 500,000 unit / l, and especially its thing made to contain by the concentration of 100 - 50,000 unit / l is desirable.

In addition, originally, the activity value of enzyme changes with activity measurement methods, and even if they are the same activity measurement method and the same enzyme, it also changes with the origins or the degrees of refining of the enzyme etc.

Therefore, it is not that the enzyme concentration (enzyme activity value) which separates from the concentration range of each enzyme indicated previously, even so the effect of this invention are not acquired.

** Substances other than enzyme If there is a substance required besides the aforementioned enzyme in a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, it will be made to contain in the reagent of this invention.

As a substance required for this reaction, adenosine triphosphate (ATP) or its salt, magnesium ion, or an oxidized type coenzyme can be mentioned, for example.

As for the concentration which makes adenosine triphosphate or its salt contain, 0.001-50g/l is desirable, and especially its 0.01-10g/l is usually desirable.

As for magnesium ion, it is [that what is necessary is just to use the thing of the form of salt with halogen ion or organic acid] usually desirable to make it contain by the concentration of 0.001 - 100mM, and especially its thing made to contain by the concentration of 0.01 - 50mM is desirable.

As an oxidized type coenzyme, nicotine amide adenine dinucleotide (oxidized type) [NAD⁺ (oxidized type)] or nicotinamide adenine dinucleotide phosphate (oxidized type) [NADP⁺ (oxidized type)] can be mentioned, for example.

4. Measurement of hydrogen peroxide or returned type coenzyme In the reagent which this invention makes a fixed quantity, as long as measurement of the hydrogen peroxide to generate or a returned type coenzyme is the method of measuring the quantity of the hydrogen peroxide generated with the aforementioned enzyme, or a returned type coenzyme, it may use what kind of method.

for example, what from the generated hydrogen peroxide or a returned type coenzyme -- it is -- the method of drawing a signal etc. can be mentioned.

In measurement of hydrogen peroxide, hydrogen peroxide itself may be measured with a hydrogen peroxide electrode etc., or another signal may be drawn from hydrogen peroxide and, more specifically, this signal may be measured.

The reaction using the Trinder system of reaction which oxidize a **** object on the basis of the existence of peroxidase (POD), it is made to generate a pigment as a method of drawing and measuring another signal from this hydrogen peroxide for example, and measures ***** of this generated pigment etc. can be mentioned.

Moreover, in measurement of a returned type coenzyme, you may measure by measuring ***** [in / in the returned type coenzyme itself / 340 etc.nm etc.] etc., or another signal may be drawn from a returned type coenzyme, and this signal may be measured.

For example, tetrazolium salt etc. can be made to be able to return to the basis of existence, such as JIAHORAZE or 1-*****_***** sulfate, a pigment can be made to be able to generate as a method of drawn and measuring another signal from this returned type coenzyme, and the reaction which measures this can be mentioned.

5. Elimination Reaction In 2nd Reagent of this Invention [the alternative reaction promoting agent which a reagent contains into the 1st reagent by consisting of the 1st reagent and the 2nd reagent] Make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride react them with the enzyme which carries out a catalyst, and by this The triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein is eliminated (decomposition).

Moreover, a reagent consists of the 1st reagent and the 2nd reagent in the 3rd reagent of this invention. The alternative reaction promoting agent contained into the 1st reagent Very low density lipoprotein, middle specific gravity lipoprotein, The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein alternatively From triglyceride, make a series of reactions which make hydrogen peroxide or a returned type coenzyme generate react them with the enzyme which carries out a catalyst, and by this Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein is eliminated (decomposition). (However, elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein is not performed.)

Although these elimination (decomposition) is performed by making it react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate a sample from triglyceride under existence of the alternative reaction promoting agent which the 1st reagent was made to contain The details about a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from this triglyceride are as having already described.

As for the hydrogen peroxide or the returned type coenzyme generated by the reaction of this elimination (decomposition), it is desirable to eliminate in the 1st step to which mix and a sample and the 1st reagent are made to react, and not to carry over to the 2nd step which mixes the 2nd reagent further.

In the case of hydrogen peroxide, the hydrogen peroxide generated in the 1st step is eliminable by making the 1st reagent contain KATARAZE or peroxidase, for example (decomposition).

The thing of animal origin, such as a thing of microbe origin, such as bacteria or mold, humans, or a cow, the thing of vegetable origin, or the thing prepared by the modifying-gene method can be used for KATARAZE, for example.

As for the concentration which makes this KATARAZE contain, it is usually desirable to carry out to more than 100 units / l.

And after eliminating the hydrogen peroxide generated by KATARAZE in the 1st step, the activity of KATARAZE is checked in the 2nd step and it is necessary to make it not work.

This can attain the substance which checks the activity of KATARAZE, such as sodium azide, by making the 2nd reagent contain etc.

Moreover, the thing of vegetable origin, such as a thing of animal origin, such as a thing of microbe origin, humans, or a cow, horseradishes, etc., such as bacteria or mold, or the thing prepared by the modifying-gene method can be used for peroxidase, for example.

As for the concentration which makes this peroxidase contain, it is usually desirable to carry out to more than 30 units / l.

In the case of a returned type coenzyme, it is eliminable by, for example, making the 1st reagent contain the dehydration enzyme which uses this returned type coenzyme as a coenzyme etc. (decomposition).

6. Composition of reagent The reagent which this invention makes a fixed quantity contains (i) alternative reaction promoting agent and the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from (ii) triglyceride.

A substance more nearly required for a series of reactions which make hydrogen peroxide or a returned type coenzyme generate than triglyceride by the aforementioned enzyme is also made to contain in the reagent of this invention.

As a substance required for this reaction, peroxidase (POD), the **** object in the Trinder system of reaction, a returned type coenzyme, or a buffer can be illustrated.

The thing of vegetable origin, such as a thing of animal origin, such as a thing of microbe origin, humans, or a cow, horseradishes, etc., such as bacteria or mold, or the thing prepared by the modifying-gene method can be used for peroxidase, for example.

As for the concentration which makes this peroxidase contain, it is usually desirable to carry out to more than 30 units / l.

As a **** object in the Trinder system of reaction, the combination of 4-amino anti PIRIN, phenol, its derivative, or a 4-amino anti PIRIN and an aniline derivative etc. can be mentioned, for example.

As for 4-amino anti PIRIN, it is usually desirable to make it contain by the concentration of 0.001-50g/l, and especially its thing made to contain by the concentration which is 0.01-10g/l is desirable.

As a derivative of phenol, 4-chloro phenol, 2, 4-dichloro phenol, 2, 4-dibromophenol or 2 and 4, 6-bird chloro phenol, or these salt can be mentioned, for example.

As an aniline derivative, for example N-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy aniline (HDAOS), N-sulfopropyl 3, 5-dimethoxy aniline (HDAPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy aniline (DAOS), N-ethyl N-sulfopropyl 3, 5-dimethoxy aniline (DAPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy 4-fluoro aniline (FDAOS), N-ethyl N-sulfopropyl 3, 5-dimethoxy 4-fluoro aniline (FDAPS), N-(2-carboxyethyl)-N-ethyl 3, 5-dimethoxy aniline (CEDB), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3-methoxyaniline (ADOS), N-ethyl N-(3-sulfopropyl)-3-methoxyaniline (ADPS), N-ethyl N-(2-hydroxy 3-sulfopropyl) aniline (ALOS), N-ethyl N-(3-sulfopropyl) aniline (ALPS), N-(3-sulfopropyl) aniline (HALPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3, 5-JIMECHIRU aniline (MAOS), N-ethyl N-(3-sulfopropyl)-3, 5-JIMECHIRU aniline (MAPS), N-ethyl N-(2-hydroxy 3-sulfopropyl)-3-methoxyaniline (TOOS), N-(2-carboxyethyl)-N-ethyl 3-methylaniline (CEMB), N-(2-carboxyethyl)-N-ethyl 3-methoxyaniline (CEMO), or these salt can be mentioned.

As for these phenol, the derivative of those, or an aniline derivative, it is usually desirable to make it contain by the concentration of 0.001-50g/l, and especially its thing made to contain by the concentration which is 0.01-10g/l is desirable.

In the reagent of this invention, as for pH at the time of mixing a sample and a reagent and performing a fixed quantity, it is desirable that it is pH five to 10 range, and it is desirable that it is especially pH 5.5 to 9.0 range. When a reagent consists of the 1st reagent and the 2nd reagent, you may set up pH of the 1st reagent and the 2nd reagent so that pH after mixing a sample and the 1st reagent and mixing the 2nd reagent further may serve as the range of the aforementioned pH.

When making a buffer contain, it is desirable to make a buffer which serves as the range of pH of the above [pH at the time of performing a fixed quantity] contain.

For example, MES, Bis-Tris, Bis-Tris propane, ADA, PIPES, ACES, MOPSO, MOPS, BES, TES, HEPES, DIPSO, TAPSO, POPSO, HEPES, HEPPSO, EPPS, Tricine, Bicine, TAPS, CHES, phosphorus acid, an orthophosphate,

boric acid, borate salt, Grishin, GURISHIRU Grishin, IMIDAZORU, or tris (hydroxymethyl) amino methane [Tris] can be mentioned.

The ground substance of the enzyme of further others [reagent / of this invention], and other enzyme, other coenzymes, The salt containing metal ions, such as alkaline metal salt or alkaline-earth-metals salt, or this, Protein, such as a chelating agent and albumin, sodium azide, an antibiotic, Or a substance in connection with elimination or influence control, a diluent base, or other reagent ingredients of the measurement interfering substance contained in samples, such as stabilizers, such as antiseptics, such as synthetic antibacteria medicine, sugars, or a high molecular compound, an activating agent, and ascorbate oxidase, etc. can be made to contain if needed suitably.

Although the thing of one reagent may be used, the reagent of this invention makes two or more reagents contain a reagent ingredient if needed, and may be constituted.

III. matter 1. specimen common to the method and reagent of this invention [a specimen] in the method and reagent of this invention As long as it tries to perform the fixed quantity of the triglyceride which the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein may exist, and is contained in very low density lipoprotein and/or middle specific gravity lipoprotein, what kind of thing may be used.

What may contain at least one sort chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein as this specimen is desirable.

What may contain very low density lipoprotein and/or middle specific gravity lipoprotein especially is desirable.

As an example of such a thing, extraction liquid [of the extraction liquid; cell or mycelia cake of **** of an extraction liquid; human or animals, such as internal organs of a humors; human or animals, such as humans or blood of an animal, serum, and plasma, or muscles,]; or vegetable extraction liquid can be mentioned.

2. Lipoprotein In this Description, main Cairo microns, very low density lipoprotein, middle specific gravity lipoprotein, low-density lipoprotein, and high density lipoprotein are indicated as lipoprotein.

However, lipoprotein other than five kinds of these main lipoprotein also exists strictly.

Therefore, let the triglyceride contained in very low density lipoprotein be triglyceride and homonymy which are contained in lipoprotein other than middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein in this Description.

Moreover, let the triglyceride contained in middle specific gravity lipoprotein be triglyceride and homonymy which are contained in lipoprotein other than very low density lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein.

And let the triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein be triglyceride and homonymy which are contained in lipoprotein other than the Cairo micron, low-density lipoprotein, and high density lipoprotein.

3. Alternative reaction promoting agent ** alternative reaction promoting agent In the method and reagent of this invention, an alternative reaction promoting agent is for making a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein in a sample.

** Illustration of an alternative reaction promoting agent The following thing of a-i is mentioned as an example of the alternative reaction promoting agent in the method and reagent of this this invention.

a) The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the thing made to react.

b) The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein from triglyceride, and the thing made to react.

Thereby, the triglyceride contained in lipoprotein other than very low density lipoprotein and/or middle specific gravity lipoprotein is eliminated.

c) The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density

lipoprotein alternatively The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and the thing made to react.

Said triglyceride contained in the lipoprotein chosen from the group which consists of very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron, low-density lipoprotein, and high density lipoprotein by this is eliminated.

(However, thing which does not perform elimination of both triglyceride contained in triglyceride and middle specific gravity lipoprotein which are contained in very low density lipoprotein.)

d) The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein from triglyceride, and the thing made to react.

Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein by this is eliminated.

e) The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in elimination processing of said d, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein from the aforementioned triglyceride and the thing made to react.

f) The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in middle specific gravity lipoprotein from triglyceride and the thing made to react.

Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein by this, and said triglyceride contained in middle specific gravity lipoprotein are eliminated.

g) The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in elimination processing of said f, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in very low density lipoprotein, and the triglyceride further contained in middle specific gravity lipoprotein depending on the case from the aforementioned triglyceride and the thing made to react.

h) The triglyceride contained in at least one sort of lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate alternatively the triglyceride contained in very low density lipoprotein from triglyceride and the thing made to react.

Said triglyceride contained in the lipoprotein chosen from the group which consists of the Cairo micron, low-density lipoprotein, and high density lipoprotein by this, and said triglyceride contained in very low density lipoprotein are eliminated.

i) The enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride in elimination processing of said h, and the Cairo micron which reacted alternatively, The triglyceride contained in low-density lipoprotein and/or high density lipoprotein, And the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate the triglyceride contained in middle specific gravity lipoprotein, and the triglyceride further contained in very low density lipoprotein depending on the case from the aforementioned triglyceride and the thing made to react.

** Type division by the reaction promotion nature of an alternative reaction promoting agent Under existence of an alternative reaction promoting agent, Very low density lipoprotein, middle specific gravity lipoprotein, the Cairo micron (henceforth [with a case] "CM"), [the triglyceride contained in each lipoprotein of low-density lipoprotein

or high density lipoprotein] When reacting with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, the type of the alternative reaction promoting agent to which the triglyceride contained in each lipoprotein can be made to react with said enzyme was shown in Table 1.

表1 各タイプ可溶化剤の各種リポ蛋白に含まれるトリグリセライドとの反応性のパターン〔計31通り〕

I. 「1種類のリポ蛋白と反応の場合」 (5通り; 1~5)

タイプ	CM	VLDL	IDL	LDL	HDL
1	CM				
2		VLDL			
3			IDL		
4				LDL	
5					HDL

II. 「2種類のリポ蛋白と反応の場合」 (10通り; 6~15)

タイプ	CM	VLDL	IDL	LDL	HDL
6	CM	VLDL			
7	CM		IDL		
8	CM			LDL	
9	CM				HDL
10		VLDL	IDL		
11		VLDL		LDL	
12		VLDL			HDL
13			IDL	LDL	
14			IDL		HDL
15				LDL	HDL

III. 「3種類のリポ蛋白と反応の場合」 (10通り; 16~25)

タイプ	CM	VLDL	IDL	LDL	HDL
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タイプ	CM	VLDL	IDL	LDL	HDL
16	CM	VLDL	IDL		
17	CM	VLDL		LDL	
18	CM	VLDL			HDL
19	CM		IDL	LDL	
20	CM		IDL		HDL
21	CM			LDL	HDL
22		VLDL	IDL	LDL	
23		VLDL	IDL		HDL
24		VLDL		LDL	HDL
25			IDL	LDL	HDL

IV. 「4種類のリポ蛋白と反応の場合」 (5通り; 26~30)

タイプ	CM	VLDL	IDL	LDL	HDL
26	CM	VLDL	IDL	LDL	
27	CM	VLDL	IDL		HDL
28	CM	VLDL		LDL	HDL
29	CM		IDL	LDL	HDL
30		VLDL	IDL	LDL	HDL

V. 「5種類のリポ蛋白と反応の場合」 (1通り; 31)

タイプ	CM	VLDL	IDL	LDL	HDL
31	CM	VLDL	IDL	LDL	HDL

As shown in this table, the type of the alternative reaction promoting agent classified according to reaction promotion nature is 31 kinds to Type 1 - Type 31.

** Directions for use of each type alternative reaction promoting agent In the method (or reagent) of this invention, it is explained below how the alternative reaction promoting agent which carried out the type division in Table 1 of the

aforementioned ** can be used.

a) In the case of the 1st method (or the 1st reagent) of this invention In the case of the 1st method (or the 1st reagent) of aforementioned this invention What is necessary is just to make the alternative reaction promoting agent of Type 10 in Table 1 exist, although a fixed quantity of triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein is carried out (or content).

Moreover, what is necessary is just to make the alternative reaction promoting agent of Type 2 exist, although a fixed quantity of triglyceride contained in very low density lipoprotein is carried out (or content).

And what is necessary is just to make the alternative reaction promoting agent of Type 3 exist, although a fixed quantity of triglyceride contained in middle specific gravity lipoprotein is carried out (or content).

In addition, when dividing a fixed quantity into two steps, the 1st step and the 2nd step, and performing it in these cases, the aforementioned alternative reaction promoting agent may be made to exist in the 1st step, and may be made to exist in the 2nd step, or may be made to exist in the 1st step and the 2nd step. The effect same in any case can be acquired.

When a fixed quantity of reagents consist of the 1st reagent and the 2nd reagent, the 1st reagent may be made to contain the aforementioned alternative reaction promoting agent, and it may be made to exist in the 2nd reagent, or the 1st reagent and the 2nd reagent may be made similarly to contain it. The effect same in any case can be acquired. Moreover, it can also be used combining two or more kinds of alternative reaction promoting agents, making it exist simultaneously (or content).

For example, making the alternative reaction promoting agent of Type 10 existing (or content) and the same effect can be acquired by making it exist combining the alternative reaction promoting agent of Type 2 of Table 1, and the alternative reaction promoting agent of Type 3 (or content).

And you may make the alternative reaction promoting agent of Type 2, and/or the alternative reaction promoting agent of Type 3 add and exist with the alternative reaction promoting agent of Type 10, although the same effect as the alternative reaction promoting agent of Type 10 is acquired for example, (or content).

b) In the case of the 2nd method (or the 2nd reagent) of this invention In the case of the 2nd method (or the 2nd reagent) of aforementioned this invention What is necessary is just to make the alternative reaction promoting agent of Type 21 in Table 1 exist in the 1st step (or the 1st reagent), although a fixed quantity of triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein is carried out (or content).

Moreover, what is necessary is just to make the alternative reaction promoting agent of Type 29 exist in the 1st step (or the 1st reagent), although a fixed quantity of triglyceride contained in very low density lipoprotein is carried out (or content).

And what is necessary is just to make the alternative reaction promoting agent of Type 28 exist in the 1st step (or the 1st reagent), although a fixed quantity of triglyceride contained in middle specific gravity lipoprotein is carried out (or content).

Moreover, although an alternative reaction promoting agent which is different from this alternative reaction promoting agent in the 2nd step (or the 2nd reagent) with the alternative reaction promoting agent made to exist in the 1st step (or the 1st reagent) (or content) may be made to exist (or content) An example of the combination of an alternative reaction promoting agent in this case is indicated below.

Although a fixed quantity of triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein is carried out The alternative reaction promoting agent of Type 21 is made to exist in the 1st step (or the 1st reagent) (or content), and what is necessary is just to make the alternative reaction promoting agent of Type 10 exist in the 2nd step (or the 2nd reagent) (or content).

In the 1st step (or after mixture of a sample and the 1st reagent), in this case, under existence of the alternative reaction promoting agent of Type 21, From triglyceride, the triglyceride contained in the Cairo micron, the triglyceride contained in low-density lipoprotein, and the triglyceride contained in high density lipoprotein react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate, and is eliminated.

The triglyceride contained in the very low density lipoprotein which remains without being eliminated here, and the

triglyceride contained in middle specific gravity lipoprotein are set to the 2nd step (or after addition of the 2nd reagent). Under existence of the alternative reaction promoting agent of Type 10, it is made to react with the enzyme which carries out the catalyst of a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, and measurement of the hydrogen peroxide to generate or a returned type coenzyme is performed.

Moreover, the alternative reaction promoting agent of Type 21 is made to exist in the 1st step (or the 1st reagent), although a fixed quantity of triglyceride contained in very low density lipoprotein is carried out (or content), and what is necessary is just to make the alternative reaction promoting agent of Type 2 exist in it in the 2nd step (or the 2nd reagent) (or content). Or the alternative reaction promoting agent of Type 29 is made to exist in the 1st step (or the 1st reagent), and what is necessary is just to make the alternative reaction promoting agent of Type 2 and/or Type 10 exist in the 2nd step (or the 2nd reagent) (or content).

And the alternative reaction promoting agent of Type 21 is made to exist in the 1st step (or the 1st reagent), although a fixed quantity of triglyceride contained in middle specific gravity lipoprotein is carried out (or content), and what is necessary is just to make the alternative reaction promoting agent of Type 3 exist in it in the 2nd step (or the 2nd reagent) (or content). Or the alternative reaction promoting agent of Type 28 is made to exist in the 1st step (or the 1st reagent) (or content), and what is necessary is just to make the alternative reaction promoting agent of Type 3 and/or Type 10 exist in the 2nd step (or the 2nd reagent) (or content).

Moreover, it can also be used combining two or more kinds of alternative reaction promoting agents, making it exist simultaneously (or content).

For example, making the alternative reaction promoting agent of Type 21 existing (or content) and the same effect can be acquired by making it exist combining the alternative reaction promoting agent of Type 1 of Table 1, and the alternative reaction promoting agent of Type 15 (or content).

And you may make the alternative reaction promoting agent of Type 1, the alternative reaction promoting agent of Type 15, etc. add and exist with the alternative reaction promoting agent of Type 28, although the same effect as the alternative reaction promoting agent of Type 28 is acquired for example, (or content).

c) In the case of the 3rd method (or the 3rd reagent) of this invention In the case of the 3rd method (or the 3rd reagent) of aforementioned this invention Although a fixed quantity of triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein is carried out What is necessary is just to make the alternative reaction promoting agent made to exist in the 1st step (the 1st reagent is made to contain), and the alternative reaction promoting agent made to exist in the 2nd step (the 2nd reagent is made to contain) exist in the combination of the type shown in Table 2 (or content).

Namely, what is necessary is just to make it exist in this table in the combination of "the type of the alternative reaction promoting agent made to exist in the 1st step (the 1st reagent is made to contain)" and "the type of the alternative reaction promoting agent made to exist in the 2nd step (the 2nd reagent is made to contain)" in the column shown by "O" (or content).

In addition, in this table, the character written in the column which showed the type of the alternative reaction promoting agent to 1-31 expresses the kind of lipoprotein containing the triglyceride in which that type of alternative reaction promoting agent promotes a reaction. namely, "V" -- very low density lipoprotein (VLDL) -- "I" -- "C" expresses middle specific gravity lipoprotein (IDL), "L" expresses the Cairo micron, and low-density lipoprotein (LDL) and "H" express high density lipoprotein (HDL) (the same also in the following Table 3 and 4).

[illegible]

表 2

第1段階に存在させる (第1試薬に含有させる) 選択的反応促進物質のタイプ																														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27				
(C)	(V)	(D)	(L)	(H)	(CV)	(CI)	(GL)	(CH)	(VI)	(VL)	(VH)	(IL)	(IH)	(LH)	(CVI)	(CVL)	(GVH)	(CIL)	(CIH)	(CLH)	(VIL)	(VILH)	(VLH)	(ILH)	(CVIL)	(GVILH)				

第 2 段階に存在させる (第 2 試薬に含有させる) 選択的反応促進物質のタイプ

For example, the alternative reaction promoting agent of Type 9 is made to exist in the 1st step (or the 1st reagent) (or content). When the alternative reaction promoting agent of Type 27 is made to exist in the 2nd step (or the 2nd reagent) (or content) and a fixed quantity is performed, In the 1st step (or after mixture of a sample and the 1st reagent), under existence of the alternative reaction promoting agent of Type 9, From triglyceride, the triglyceride contained in the Cairo micron and the triglyceride contained in high density lipoprotein react with the enzyme which

Namely, what is necessary is just to make it exist in this table in the combination of "the type of the alternative reaction promoting agent made to exist in the 1st step (the 1st reagent is made to contain)" and "the type of the alternative reaction promoting agent made to exist in the 2nd step (the 2nd reagent is made to contain)" in the column shown by "O" (or content).

[illegible]

第1段階に存在させる(第1試薬に含有させ

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	(C)	(V)	(O)	(L)	(H)	(GV)	(GI)	(CL)	(CH)	(VI)	(VL)	(VH)	(IL)	(IH)	(LH)	(GV)	(GVL)	(GVH)	(GI)
1 (C)	○		○	○	○		○	○	○				○	○	○				
2 (V)		○																	
3 (O)			○	○	○		○	○	○				○	○	○				
4 (L)																			
5 (H)																			
6 (GV)	○						○	○	○										○
7 (GI)																			
8 (CL)																			
9 (CH)																			
10 (VI)			○				○						○	○					○
11 (VL)				○			○						○		○				○
12 (VH)					○				○					○	○				
13 (IL)																			
14 (IH)																			
15 (LH)																			○
16 (GV)							○												○
17 (GVL)								○											○
18 (GVH)									○										
19 (GIL)																			
20 (GIH)																			
21 (CLH)																			
22 (VIL)													○						○
23 (VIH)														○					
24 (VLH)															○				
25 (ILH)																			○
26 (CVIL)																			
27 (GVILH)																			
28 (CVLH)																			
29 (CILH)																			
30 (VILH)																			
31 (CVILH)																			

第2段階に存在させる(第2試薬に含有させる)選択的反応促進物質のタイプ

Namely, what is necessary is just to make it exist in this table in the combination of "the type of the alternative reaction promoting agent made to exist in the 1st step (the 1st reagent is made to contain)" and "the type of the alternative reaction promoting agent made to exist in the 2nd step (the 2nd reagent is made to contain)" in the column shown by "O" (or content).

[illegible]

[illegible]

Also in the 3rd method (or the 3rd reagent) of the above this invention, it can also be used combining two or more kinds of alternative reaction promoting agents, making it exist simultaneously (or content).

For example, making the alternative reaction promoting agent of Type 15 existing (or content) and the same effect can be acquired by making it exist combining the alternative reaction promoting agent of Type 4 of Table 1, and the alternative reaction promoting agent of Type 5 (or content).

And you may make the alternative reaction promoting agent of Type 1, the alternative reaction promoting agent of Type 15, etc. add and exist with the alternative reaction promoting agent of Type 28, although the same effect as the

alternative reaction promoting agent of Type 28 is acquired for example, (or content).

**** Example of an alternative reaction promoting agent** In the method (reagent of this invention) of this invention, a surface-active agent, polyoxyalkylene, its derivative, polysaccharide, or its derivative can be mentioned as an example of an alternative reaction promoting agent.

As a surface-active agent, a nonionic surface-active agent, a cationic surfactant, a negative ion nature surface-active agent, or an ampholytic surface active agent can be mentioned.

As a nonionic surface-active agent, for example Polyoxyalkylene poly oars Polyoxyethylene alkyl ether, a polyoxy ethylene alkylphenyl formaldehyde condensation thing, Polyoxyethylene alkyl phenyl ether or n-Cheb ****- beta-D-CHIOGURUKOSHIDO (n-Cheb ****- beta-D-CHIOGURUKOPIRANOSHIDO, n-Heptyl-beta-D-thioglucofuranoside) can be mentioned.

The number of addition Mol of polyoxyalkylene in a polyoxyalkylene poly oar etc. has the desirable range of 5-1,000, and especially its range of 10-500 is desirable.

Moreover, the number of addition Mol of the ethylene oxide in polyoxyethylene alkyl ether or a polyoxy ethylene alkylphenyl formaldehyde condensation thing has the desirable range of 5-1,000, and especially its range of 5-500 is desirable.

And the number of addition Mol of the ethylene oxide in polyoxyethylene alkyl phenyl ether has the desirable range of 5-1,000, and especially its range of 5-500 is desirable.

As an ampholytic surface active agent, it is 3-[(3-Cholamidopropyl) dimethylammonio]-2-hydroxypropanesulfonic, for example. acid (CHAPSO) etc. can be mentioned.

Moreover, as polyoxyalkylene or its derivative, polyoxy ethylene (polyethylene glycols), its derivative, polyoxypropylene (polypropylene glycol), or its derivative can be mentioned, for example.

The number of addition Mol of this polyoxyalkylene has the desirable range of 5-1,000, and especially its range of 10-500 is desirable.

As polysaccharide or its derivative, cyclodextrin or its derivative, dextran sulfate or its derivative, dextran or its derivative, heparin, or its derivative can be mentioned, for example.

alpha-cyclodextrin, beta-cyclodextrin, or gamma-cyclodextrin can be mentioned as cyclodextrin.

As a cyclodextrin derivative, for example Moreover, alpha-cyclodextrin, The hydroxyl group of beta-cyclodextrin or gamma-cyclodextrin can mention the things replaced by the hydroxypropyl machine, the MARUTOSHIRU machine, the hydroxy butyl group, the diethylamino ethyl group, etc., these cyclodextrin, or the bridge construction thing of the derivative.

The thing of a molecular weight of the range of 1,000-5,000,000 is desirable, and dextran sulfate or its derivative has [a molecular weight] especially the desirable thing of the range of 5,000-1,000,000.

The more detailed example of these alternative reaction promoting agents was shown in Table 5.

表 5

濃度	選択的反応促進物質	販売元	化学構造
0.1%	TMH-7EX	日光ケミカルズ	POE(7)トリメチルヘキシルエーテル
0.1%	サンニックスFA-103	三洋化成工業	特殊ポリオール
0.1%	PEG2,000	和光純薬工業	ポリエチレングリコール2,000
0.1%	PEG6,000	和光純薬工業	ポリエチレングリコール6,000
0.1%	PEG10,000	日光ケミカルズ	ポリエチレングリコール10,000
0.1%	PEG20,000	和光純薬工業	ポリエチレングリコール20,000
0.1%	PEG1,540	和光純薬工業	ポリエチレングリコール1,500
0.1%	エマルゲン911	花王	ポリオキシエチレンアルキルエーテル
0.1%	KF-354	信越シリコン	ポリエーテル変性シリコンオイル
0.1%	KF-907	信越シリコン	ポリエーテル変性シリコンオイル
0.1%	サルコシネートCN-100	日光ケミカルズ	ココイルサルコシンナトリウム
0.1%	NP-10	日光ケミカルズ	POE(10)ノニルフェニルエーテル
0.1%	BL-9EX	日光ケミカルズ	POE(9)ラウリルエーテル
0.1%	KF-351	信越シリコン	ポリエーテル変性シリコンオイル
0.1%	KF-700	信越シリコン	親水性特殊変性シリコンオイル
0.1%	R-1020	日光ケミカルズ	POEノニルフェニルホルムアルデヒド縮合物
0.1%	サンニックスGP400	三洋化成工業	ポリオキシプロピル化グリセリン
0.1%	ブルロニックP-85	旭電化工業	ポリオキシエチレン・ポリオキシプロピレン縮合物
0.1%	ブルロニックL-34	旭電化工業	ポリオキシエチレン・ポリオキシプロピレン縮合物
0.1%	ブルロニックL-44	旭電化工業	ポリオキシエチレン・ポリオキシプロピレン縮合物
0.1%	アデカトールSO-120	旭電化工業	高級アルコールエトキシレート
0.1%	アデカトールNP-720	旭電化工業	ポリオキシエチレンアルキルアリルエーテル
0.1%	PEG1,000	和光純薬工業	ポリエチレングリコール1,000
0.5%	OP-8	日光ケミカルズ	POE(8)オクチルフェニルエーテル
0.1%	BT-7	日光ケミカルズ	POE(7)2級アルキルエーテル
0.1%	BT-9EX	日光ケミカルズ	POE(9)2級アルキルエーテル
0.1%	Tween20	和光純薬工業	POE(20)ソルビタンモノラウレート
0.1%	OP-10	日光ケミカルズ	POE(10)オクチルフェニルエーテル
0.1%	POE-p-トルエンスルホアミド	日光ケミカルズ	POE-p-トルエンスルホアミド
0.1%	デキストラン硫酸 ~500,000	ファルマシア	デキストラン硫酸
0.1%	α -CD	和光純薬工業	α -シクロデキストリン
0.1%	ヒドロキシブチル- α -CD		ヒドロキシブチル- α -シクロデキストリン
0.1%	マルトシル- β -CD	横浜国際バイオ研究所	マルトシル- β -シクロデキストリン
0.1%	ヒドロキシブチル- β -CD		ヒドロキシブチル- β -シクロデキストリン
0.1%	ヒドロキシプロピル- β -CD		ヒドロキシプロピル- β -シクロデキストリン
0.1%	水溶性 β -シクロデキストリンポリマー		水溶性 β -シクロデキストリンポリマー (エピクロルヒドリン架橋)
0.1%	ジエチルアミノエチル- β -CD		ジエチルアミノエチル- β -シクロデキストリン
0.1%	γ -CD		γ -シクロデキストリン
0.1%	ヒドロキシプロピル- α -CD		ヒドロキシプロピル- α -シクロデキストリン
0.1%	ヒドロキシプロピル- γ -CD		ヒドロキシプロピル- γ -シクロデキストリン

POE: ポリオキシエチレン

You may make an alternative reaction promoting agent exist combining two or more kinds of things, as stated previously (or content).

[the concentration in which an alternative reaction promoting agent is made to exist (or content)] The kind and the origin of enzyme which carry out the catalyst of the kind of alternative reaction promoting agent, and a series of reactions which make hydrogen peroxide or a returned type coenzyme generate from triglyceride, Since it changes with the concentration of the triglyceride contained in the lipoprotein in a sample, or mixed ratios of the 1st reagent and the 2nd reagent Although what is necessary is just to make it exist generally by ***** and the concentration which was suitable for the condition suitably (or content), it is [that what is necessary is just to make it exist by

0.001 to 10% of concentration (or content)] usually desirable to make it exist by 0.01 to 5% of concentration (or content).

4. Reaction auxiliary substance You may make a reaction auxiliary substance exist in the method (reagent of this invention) of this invention with an alternative reaction promoting agent (or content).

Existence (or content) of this reaction auxiliary substance can raise work of the promotion of an alternative reaction of an alternative reaction promoting agent.

As an example of a reaction auxiliary substance, poly ANION, halogen ion, a metal ion, or REKUCHIN can be mentioned.

As poly ANION, phosphorus tungsten etc. can be mentioned, for example.

As halogen ion, crawl ion etc. can be mentioned, for example.

As a metal ion, divalent metallic ions, such as copper ion or manganese ion, etc. can be mentioned, for example.

As REKUCHIN, lentil REKUCHIN etc. can be mentioned, for example.

You may make these reaction auxiliary substances exist combining two or more kinds of things (or content).

What is necessary is just to make it exist generally by ***** and the concentration which was suitable for the condition suitably, since the concentration in which a reaction auxiliary substance is made to exist (or content) changes with various conditions like the case of an alternative reaction promoting agent (or content).

This Description includes the contents indicated on Tokuganhei 11-128994 and the Description of PCT/JP 99/No. 06723, and/or Drawings which are the foundation of the preference of an application concerned.

The best form for inventing Next, although a working example is given and this invention is explained concretely, this invention is not limited to these.

[Working example 1] Fixed-quantity-1 of bird GUSERAIDO in a refining lipoprotein drawing part with the method and reagent of this invention The method and reagent of this invention using n-Cheb *****- beta-D-CHIOGURUKOSHIDO as an alternative reaction promoting agent performed the fixed quantity of triglyceride in a refining lipoprotein drawing part.

As the 1st reagent, glycerol kinase 1.0 unit / ml, glycerol 3-phosphorus acid oxidase 8.0 unit / ml, KATARAZE, adenosine 3-phosphorus acid 4.1 mmol/l, N-(3, 5-dimethoxy phenyl)-N'-succinyl ethylene diamine sodium 0.94 mmol/l, good buffer solution (pH 6.0) was set n-Cheb *****- beta-D-CHIOGURUKOSHIDO 0.4% -- thing preparation was carried out.

Moreover, as the 2nd reagent, lipoprotein lipase 2.0 unit / ml, peroxidase, 4-amino anti PIRIN 2.5 mmol/l, and the thing that set good buffer solution were prepared.

In actual measurement, put 3micro of serum 1, and 300micro of the 1st reagent 1 into the test tube which was 37 degrees C, it was made to react for 5 minutes, and then 100micro of the 2nd reagent 1 was added.

***** of reaction liquid was measured on the wavelength of 600nm immediately after that and 5 minutes afterward.

Based on this value, the triglyceride value was computed using the analytical curve created beforehand.

On the other hand, blood was extracted with the blood collecting pipe into which the solidification prevention agent was put, and four kinds of lipoprotein of the Cairo micron, very low density lipoprotein and middle specific gravity lipoprotein, low-density lipoprotein, and high density lipoprotein was separated with the density gradient centrifugation method.

From these four samples, a fixed quantity by this method and the fixed quantity of the total triglyceride by a commercial reagent kit (DETAMINA LTG-II; made by Kyowa Medex, Inc) were performed, and it asked for both ratio.

This result is shown in drawing 1 .

It is shown by this invention that the reaction decomposition of the triglyceride in the Cairo micron, low-density lipoprotein, and high density lipoprotein can be carried out alternatively.

[Working example 2] Fixed-quantity-2 of bird GUSERAIDO in a refining lipoprotein drawing part with the method and reagent of this invention The method and reagent of this invention performed the fixed quantity of triglyceride in a refining lipoprotein drawing part using various kinds of alternative reaction promoting agents.

1. Manufacture (1) of reagent of this invention Manufacture of the 1st reagent (A) The following reagent ingredient

was dissolved in pure water so that it might become the concentration of a description, respectively, and pH 6.0 (20 degrees C) reagent was prepared.

A reagent ingredient Concentration 2-morpholino ethane sulfonic acid [MES] 50 mmol/lN-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy aniline sodium [HDAOS] (**** object) 1.5 mmol/l glycerol kinase 150 unit / l glycerol 3-phosphorus acid oxidase 3,000 units / l adenosine triphosphate sodium 0.5 mmol/l chlorination magnesium and a 6 hydration thing 1 mmol/l KATARAZE 100,000 units / l reaction promoting agent [substance name and concentration are written] to Table 6.

(2) Manufacture of the 2nd reagent (B) The following reagent ingredient was dissolved in pure water so that it might become the concentration of a description, respectively, and pH 6.0 (20 degrees C) reagent was prepared.

A reagent ingredient Concentration 2-morpholino ethane sulfonic acid [MES] 50 mmol/l4-amino anti PIRIN 0.75 mmol/l peroxidase 600 units / l lipoprotein lipase 120,000 units / l sodium azide 0.1% reaction promoting agent [substance name and concentration are written] to Table 6.

2. Manufacture of the 1st reagent (C) of manufacture (1) of total triglyceride fixed-quantity reagent (contrast) The following reagent ingredient was dissolved in pure water so that it might become the concentration of a description, respectively, and pH 6.0 (20 degrees C) reagent was prepared.

A reagent ingredient Concentration 2-morpholino ethane sulfonic acid [MES] 50 mmol/lN-(2-hydroxy 3-sulfopropyl)-3, 5-dimethoxy aniline sodium [HDAOS] (**** object) 1.5 mmol/l glycerol kinase 150 unit / l glycerol 3-phosphorus acid oxidase 3,000 units / l adenosine triphosphate sodium 0.5 mmol/l chlorination magnesium and a 6 hydration thing 1 mmol/l KATARAZE 100,000 units / l ADEKANORU B-795 (Asahi Denka Kogyo) 0.5% (w/v)

(2) Manufacture of the 2nd reagent (D) The following reagent ingredient was dissolved in pure water so that it might become the concentration of a description, respectively, and pH 6.0 (20 degrees C) reagent was prepared.

A reagent ingredient Concentration 2-morpholino ethane sulfonic acid [MES] 50 mmol/l4-amino anti PIRIN 0.75 mmol/l peroxidase 600 units / l lipoprotein lipase 120,000 units / l sodium azide 0.1% ADEKANORU B-795 (Asahi Denka Kogyo) 0.5% (w/v)

3. Manufacture for refining lipoprotein drawing A part for the drawing of the lipoprotein from which five kinds of specific gravity, the Cairo micron, very low density lipoprotein, middle specific gravity lipoprotein, low-density lipoprotein, and high density lipoprotein, differs was obtained using the density gradient centrifugation method, respectively like the working example 1. A fixed quantity was presented with five kinds of these drawings as a sample.

4. Fixed quantity of triglyceride in lipoprotein drawing part The procedure shown below performed measurement of triglyceride in a lipoprotein drawing part using Hitachi 7150 type automatic analysis equipment (Hitachi).

To 3microl for each lipoprotein drawing prepared by said 3, 1st (reagent A) 250microl of said this invention of (1) of 1 was added, and it warmed for 5 minutes at 37 degrees C to it.

5 minutes after the 1st (reagent A) addition of this invention, said 2nd (reagent B) 125microL of (2) of 1 was added. 37 degrees C of this reaction **** and ***** of 5 minutes after were measured by two-wave analysis with a

dominant wavelength of 600nm, and a subwavelength of 700nm.

In addition, ***** made the value which deducted ***** measured by the same method using a physiological saline as a sample from ***** measured by the aforementioned method using a part for each lipoprotein drawing as a sample the measured value of *****.

The fixed quantity of triglyceride in each lipoprotein drawing part prepared by said 3 was performed like the aforementioned method using the 1st reagent (C) of said total triglyceride fixed-quantity reagent of (1) of 2, and the 2nd reagent (D) of said total triglyceride fixed-quantity reagent of (2) of 2.

In order to confirm the effect of each alternative reaction promoting agent which the reagent of this invention was made to contain, the value which *(ed) the measured value of ***** when using the reagent (A and B) of this invention by the measured value of ***** when using the total triglyceride fixed-quantity reagent (C and D) was calculated.

This value was shown in Table 6.

表 6

濃度	選択的反応促進物質	試料(リポ蛋白画分)					タイプ
		CM	VLDL	IDL	LDL	HDL	
0.5%	トリグリセリド定量試薬(対照)	1.00	1.00	1.00	1.00	1.00	—
0.1%	TMH-7EX	0.09	0.31	0.20	0.04	0.24	23
0.5%		0.98	1.04	1.17	1.22	1.14	31
0.1%	BT-7	1.02	1.02	1.09	1.20	1.10	31
0.1%	サンニクスFA-103	0.78	0.66	0.43	0.03	0.08	16
0.2%		0.17	0.99	0.54	0.26	0.13	10
0.4%		0.20	1.05	0.69	0.45	0.22	10
0.5%		0.27	1.00	0.56	0.12	0.24	10
0.6%		0.30	1.04	0.81	0.51	0.32	10
0.8%		0.53	1.05	0.90	0.62	0.40	26
1.0%		0.92	1.03	1.04	1.03	1.03	31
0.5%	PEG2,000	0.54	0.29	0.47	0.05	0.11	16
0.1%	PEG6,000	0.59	0.27	0.40	0.05	0.06	7
0.5%		0.76	0.27	0.44	0.04	0.07	7
0.1%	PEG10,000	0.76	0.27	0.46	0.07	0.08	7
0.5%		0.47	0.29	0.51	0.04	0.14	16
0.1%	PEG20,000	0.24	0.26	0.49	0.07	0.15	10
0.5%		0.80	0.27	0.45	0.04	0.06	7
0.1%	PEG1,540	0.81	0.30	0.44	0.09	0.08	7
0.5%		0.84	0.30	0.47	0.07	0.07	7
0.1%	エマルゲン911	0.05	0.18	0.28	0.71	1.01	15
0.5%		0.05	0.24	0.51	0.93	1.01	25
0.1%	サルコシネートCN-100	0.84	0.92	0.38	0.05	0.04	6
0.5%		0.82	0.96	0.30	0.01	0.04	6
0.1%	NP-10	0.18	1.00	1.03	1.11	1.08	30
0.5%		0.22	1.00	1.08	1.08	1.08	30
0.1%	BL-9EX	0.07	0.23	0.41	0.83	1.08	15
0.1%	KF-351	0.17	0.17	0.36	0.09	0.30	14
0.1%	KF-700	0.81	0.25	0.32	0.03	0.06	1
0.1%	R-1020	0.02	0.07	0.10	0.32	0.93	5
0.5%		0.02	0.08	0.15	0.37	1.03	5
0.1%	サンニクスGP400	0.06	0.26	0.26	0.12	0.30	23
0.5%		0.14	0.43	0.61	0.32	0.58	30
0.1%	ブルロニックP-85	0.01	0.02	0.00	0.03	0.35	5
0.1%	ブルロニックL-34	0.71	0.30	0.54	0.11	0.23	7
0.1%	ブルロニックL-44	0.52	0.22	0.36	0.07	0.17	7
0.5%		0.14	0.23	0.31	0.03	0.32	23
0.1%	アデカールSO-120	0.79	1.05	1.05	1.22	1.15	31
0.1%	アデカールNP-720	0.03	0.11	0.10	0.51	0.93	15
0.5%		0.03	0.13	0.13	0.64	0.96	15
0.1%	PEG1,000	0.82	0.24	0.52	0.00	0.00	7
0.5%	OP-8	1.01	1.08	1.07	1.17	1.08	31
0.1%	BT-9EX	0.99	1.07	1.10	1.14	1.06	31
0.5%		1.03	1.05	1.15	1.17	1.11	31
0.1%	Tween20	1.13	1.08	1.07	1.63	0.60	26
0.5%		0.97	1.02	1.03	1.09	0.83	31
0.1%	OP-10	0.08	0.59	0.78	1.11	1.04	30
0.5%		0.10	0.70	0.93	1.16	1.11	30
0.1%	POE-p-トルエンスルホアミド	0.76	0.21	0.38	0.05	0.11	7
0.5%		0.27	0.33	0.26	-0.03	0.11	16
0.1%	デキストラン硫酸 ~500,000	0.12	0.42	0.17	0.07	0.14	2
0.5%		0.14	0.86	0.19	0.10	0.14	2
0.1%	α -CD	0.33	0.50	0.38	0.08	0.06	16
0.5%		0.63	0.75	0.75	0.30	0.06	16
0.1%	ヒドロキシブチル- α -CD	0.35	0.55	0.40	0.09	0.06	16
0.5%		0.57	0.68	0.58	0.14	0.05	16
0.1%	マルトシル- β -CD	0.33	0.63	0.34	0.05	0.05	16
0.5%		0.40	0.52	0.34	0.07	0.05	16
0.1%	ヒドロキシブチル- β -CD	0.44	0.64	0.43	0.08	0.05	16
0.5%		0.73	0.72	0.61	0.21	0.04	16
0.1%	ヒドロキシプロピル- β -CD	0.36	0.71	0.41	0.10	0.06	16
0.5%		0.46	0.58	0.45	0.09	0.04	16
0.1%	水溶性 β -シクロデキストリンポリマー	0.44	0.56	0.46	0.10	0.05	16
0.1%	ジエチルアミノエチル- β -CD	0.29	0.66	0.37	0.08	0.06	10
0.5%		0.37	0.50	0.35	0.07	0.05	16
0.1%	γ -CD	0.32	0.97	0.34	0.06	0.05	2
0.5%		0.53	1.03	0.59	0.11	0.06	16
0.1%	ヒドロキシプロピル- α -CD	0.22	0.59	0.32	0.07	0.05	10
0.5%		0.35	0.52	0.37	0.07	0.04	16
0.1%	ヒドロキシプロピル- γ -CD	0.31	0.95	0.34	0.10	0.06	2
0.5%		0.46	1.02	0.48	0.08	0.06	2

CM: カイロミクロン
VLDL: 超低比重リポ蛋白
IDL: 中間比重リポ蛋白
LDL: 低比重リポ蛋白
HDL: 高比重リポ蛋白

0.1%	0.5%	0.31	0.95	0.34	0.10	0.06	2	LDL: 低比重リポ蛋白
0.1%	0.5%	0.46	1.02	0.48	0.08	0.06	2	HDL: 高比重リポ蛋白

The alternative reaction promoting agent (Type 1) to which only the triglyceride contained in the Cairo micron is made to react from the result of Table 6 is KF-700. It was 0.1%.

The alternative reaction promoting agent (Type 2) to which only the triglyceride contained in very low density lipoprotein is made to react is dextran sulfate -500,000. 0.1 and 0.5%, gamma-CD 0.1% and hydroxypropyl gamma-CD They were 0.1 and 0.5%.

The alternative reaction promoting agent (Type 5) to which only the triglyceride contained in high density lipoprotein is made to react is R-1020. 0.1, 0.5%, and Pluronic P-85 It was 0.1%.

The alternative reaction promoting agent (Type 6) to which the triglyceride contained in the Cairo micron and very low density lipoprotein is made to react is sarcosinate CN-100. They were 0.1 and 0.5%.

[the alternative reaction promoting agent (Type 7) to which the triglyceride contained in the Cairo micron and middle specific gravity lipoprotein is made to react] PEG1,000 0.1%, PEG1,540 0.1 and 0.5%, PEG6,000 0.1 and 0.5%, PEG10,000 0.1%, PEG20,000 0.5%, Pluronic L-34 0.1%, Pluronic L-44 0.1% and POE-p-toluene sulfo amide It was 0.1%.

[the alternative reaction promoting agent (Type 10) to which the triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein is made to react] San Knicks FA-103 0.2, 0.4, 0.5 and 0.6%, PEG20,000 0.1%, diethylamino ethyl beta-CD 0.1% and hydroxypropyl alpha-CD It was 0.1%.

The alternative reaction promoting agent (Type 14) to which the triglyceride contained in middle specific gravity lipoprotein and high density lipoprotein is made to react is KF-351. It was 0.1%.

The alternative reaction promoting agent (Type 15) to which the triglyceride contained in low-density lipoprotein and high density lipoprotein is made to react is emulgen 911. 0.1%, BL-9EX 0.1% and ADEKATORU NP-720 They were 0.1 and 0.5%.

[the alternative reaction promoting agent (Type 16) to which the triglyceride contained in the Cairo micron, very low density lipoprotein, and middle specific gravity lipoprotein is made to react] San Knicks FA-103 0.1%, PEG2,000 0.5%, PEG10,000 0.5%, POE-p-toluene sulfo amide 0.5%, Alpha-CD, hydroxy butyl alpha-CD, malto ****- beta-CD, hydroxy butyl beta-CD, and 0.1 and 0.5% of hydroxypropyl beta-CD Water-soluble beta-cyclodextrin polymer They were diethylamino ethyl beta-CD, gamma-CD, and 0.5% of hydroxypropyl alpha-CD 0.1%.

The alternative reaction promoting agent (Type 23) to which the triglyceride contained in very low density lipoprotein, middle specific gravity lipoprotein, and high density lipoprotein is made to react is TMH-7EX0.1% and San Knicks GP-400. 0.1% and Pluronic L-44 It was 0.5%.

The alternative reaction promoting agent (Type 25) to which the triglyceride contained in middle specific gravity lipoprotein, low-density lipoprotein, and high density lipoprotein is made to react was emulgen 9110.5%.

The alternative reaction promoting agent (Type 26) to which the triglyceride contained in the Cairo micron, very low density lipoprotein, middle specific gravity lipoprotein, and low-density lipoprotein is made to react is Tween20. 0.1% and San Knicks FA-103 It was 0.8%.

[the alternative reaction promoting agent (Type 30) to which the triglyceride contained in very low density lipoprotein, middle specific gravity lipoprotein, low-density lipoprotein, and high density lipoprotein is made to react] NP-10 0.1 and 0.5%, and San Knicks GP-400 0.5% and OP-10 They were 0.1 and 0.5%.

The Cairo micron, very low density lipoprotein, middle specific gravity lipoprotein, low-density lipoprotein, [and the alternative reaction promoting agent (Type 31) reacted to the triglyceride contained in all the lipoprotein of high density lipoprotein] TMH-7EX 0.5% and BT-7 0.1% and ADEKATORU SO-120 0.1% and OP-8 0.5%, BT-9EX 0.1 and 0.5%, Tween20 0.5% and San Knicks FA-103 It was 1%.

In how to make this invention a fixed quantity from these results, and the reagent made a fixed quantity It was confirmed in the alternative reaction promoting agent independent or that the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein by combining and making it exist or contain can be alternatively made a fixed quantity.

[namely, each alternative reaction promoting agent with which it inquired here and the type as an alternative reaction

promoting agent became clear] By making it exist or contain and performing a fixed quantity according to a description of previous "directions for use of ** each type alternative reaction promoting agent" ("3. alternative reaction promoting agent" of "the matter common to the method and reagent of this invention" of "the form of implementation of invention") The triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein can be alternatively made a fixed quantity.

If an example is given, will make the triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein react. San Knicks FA-103 which is the alternative reaction promoting agent of Type 10 0.2, 0.4, 0.5, or 0.6%, PEG20, 0000.1%, diethylamino ethyl beta-CD 0.1% or hydroxypropyl alpha-CD By making 0.1% exist or contain shows that the triglyceride contained in very low density lipoprotein and middle specific gravity lipoprotein can be alternatively made a fixed quantity.

[Working example 3] Fixed-quantity-3 of bird GUSERAIDO in a refining lipoprotein drawing part with the method and reagent of this invention The method and reagent of this invention using San Knicks FA-103 as an alternative reaction promoting agent performed the fixed quantity of triglyceride in a refining lipoprotein drawing part.

1. Manufacture of 1st Reagent of San Knicks FA-103 Content as an Alternative Reaction Promoting Agent Except having made the content concentration into 0.2, 0.4, 0.6, 0.8, or 1.0% (w/v) using San Knicks FA-103 It prepared as the same reagent ingredient as the 1st reagent (A) and concentration of 1 of this invention, and five kinds of 1st reagent of San Knicks FA-103 content was prepared. [of said working example 2] [of (1)]

2. Manufacture of 2nd Reagent of San Knicks FA-103 Content as an Alternative Reaction Promoting Agent Except having made the content concentration into 0.2, 0.4, 0.6, 0.8, or 1.0% (w/v) using San Knicks FA-103 It prepared as the same reagent ingredient as the 2nd reagent (B) and concentration of 1 of this invention, and five kinds of 2nd reagent of San Knicks FA-103 content was prepared. [of said working example 2] [of (2)]

Five kinds of lipoprotein drawings prepared by the same technique as 3 of said working example 2 were made into the sample, and the fixed quantity of triglyceride in each lipoprotein drawing part was performed like 4 of said working example 2 using each 1st reagent of Sun Knicks FA-103 content, and the 2nd reagent.

Moreover, the fixed quantity of triglyceride in each lipoprotein drawing part was performed like a fixed quantity of these methods using the 1st reagent [of said working example 2] of 2 of the total triglyceride fixed-quantity reagent of (1) (C), and the 2nd reagent [of said working example 2] of 2 of the total triglyceride fixed-quantity reagent of (2) (D).

In order to confirm the effect of each alternative reaction promoting agent which the San Knicks FA-103 content reagent of this invention was made to contain, the value which ** (ed) the measured value of ***** when using a San Knicks FA-103 content reagent by the measured value of ***** when using the total triglyceride fixed-quantity reagent (C and D) was calculated.

This value was shown in Table 7 and drawing 2 .

[in addition, the measured value of ***** when a vertical axis uses a San Knicks FA-103 content reagent in this figure] Expressing the value which ** (ed) by the measured value of ***** when using the total triglyceride fixed-quantity reagent, a horizontal axis expresses the concentration [% (w/v)] of San Knicks FA-103 the San Knicks FA-103 content reagent of this invention was made to contain.

表 7

濃度%	選択的反応促進物質	試料(リポ蛋白画分)				
		CM	VLDL	IDL	LDL	HDL
	総トリグリセライド測定試薬(対照)	1.00	1.00	1.00	1.00	1.00
0.2	サンニックスFA-103	0.17	0.99	0.54	0.26	0.13
0.4		0.20	1.05	0.69	0.45	0.22
0.6		0.30	1.04	0.81	0.51	0.32
0.8		0.53	1.05	0.90	0.62	0.40
1		0.92	1.03	1.04	1.03	1.03

CM: カイロミクロン
VLDL: 超低比重リポ蛋白
IDL: 中間比重リポ蛋白
LDL: 低比重リポ蛋白
HDL: 高比重リポ蛋白

Table 7 and drawing 2 show that the concentration of San Knicks FA-103 can make a fixed quantity alternatively very low density lipoprotein and/or middle specific gravity lipoprotein among lipoprotein by existence (content) of this substance in the concentration up to 0.8% (w/v).

Selectivity is remarkable in 0.2% (w/v) of concentration in particular.

Rather than the above result, it was confirmed that how to make this invention a fixed quantity and the reagent made a fixed quantity can make a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein.

In addition, in this example, the reaction time course of the San Knicks FA-103 content reagent [Hitachi 7150 type automatic analysis equipment of this invention when San Knicks FA-103 content concentration carries out parts for a fixed quantity of each lipoprotein drawing using 0.4% (w/v) of thing] was shown in drawing 3.

Moreover, in said working example 2, the reaction time course of Hitachi 7150 type automatic analysis equipment when carrying out parts for a fixed quantity of each lipoprotein drawing using the total triglyceride fixed-quantity reagent (C and D) was shown in drawing 4.

In these figures, a vertical axis shows ***** (the dominant wavelength of 600nm, subwavelength of 700nm). Moreover, a horizontal axis shows the light measurement point of this analysis equipment, and is measuring for [reaction time] about 10 minutes by 50 points.

In drawing 4, there is no selectivity over triglyceride in lipoprotein, and carrying out a fixed quantity of triglyceride contained in all the lipoprotein is shown in the total triglyceride fixed-quantity reagent at it.

By drawing 3, the triglyceride contained in very low density lipoprotein among lipoprotein has reacted alternatively to it. It is shown that San Knicks FA-103 are the alternative reaction promoting agent which the triglyceride contained in very low density lipoprotein can be made to be able to react alternatively, and can make it a fixed quantity.

It turns out that how to make a fixed quantity this invention which an alternative reaction promoting agent is made to exist, or contains it also from these figures, and the reagent made a fixed quantity can make a fixed quantity alternatively the triglyceride contained in very low density lipoprotein and/or middle specific gravity lipoprotein. It shall put in for this Description as it is by referring to all the publications quoted in this Description, a patent, and patent application.

Possibility of industrial use [the method and reagent which make a fixed quantity alternatively the triglyceride contained in the very low density lipoprotein and/or middle specific gravity lipoprotein of this invention]

Complicated pre-disposal, such as centrifugality and precipitation, is unnecessary, it can apply to the automatic analysis equipment for clinical examinations, and useful data can be obtained simple and correctly for prevention and medical treatment of arteriosclerosis.

[Brief Description of the Drawings]

Drawing 1 is the figure having shown the result when performing the fixed quantity of the triglyceride contained in four kinds of lipoprotein drawings, using n-Cheb ****- beta-D-CHIOGURUKOSHIDO as an alternative reaction promoting agent.

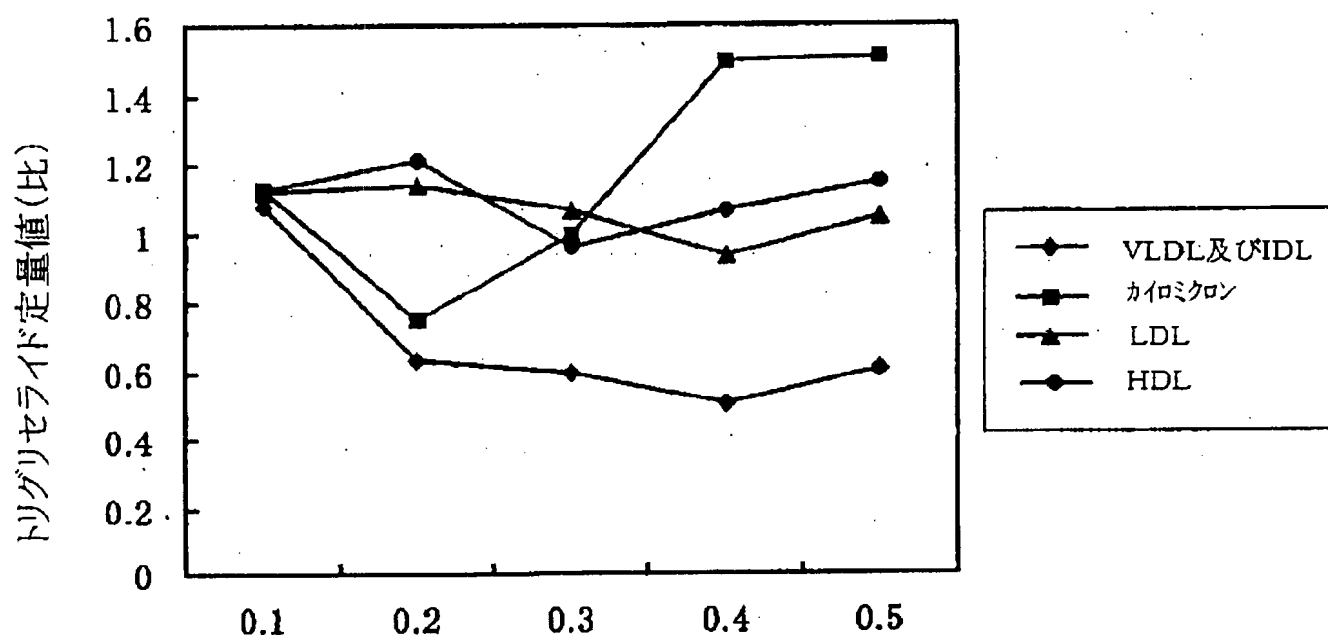
Drawing 2 is the figure having shown the result when performing the fixed quantity of the triglyceride contained in five kinds of lipoprotein drawings, using San Knicks FA-103 as an alternative reaction promoting agent.

Drawing 3 is the figure having shown the reaction time course of Hitachi 7150 type automatic analysis equipment when performing the fixed quantity of the triglyceride contained in five kinds of lipoprotein drawings, using San Knicks FA-103 as an alternative reaction promoting agent.

Drawing 4 is the figure having shown the reaction time course of Hitachi 7150 type automatic analysis equipment when the total triglyceride fixed-quantity reagent performs the fixed quantity of the triglyceride contained in five kinds of lipoprotein drawings.

[Drawing 1]

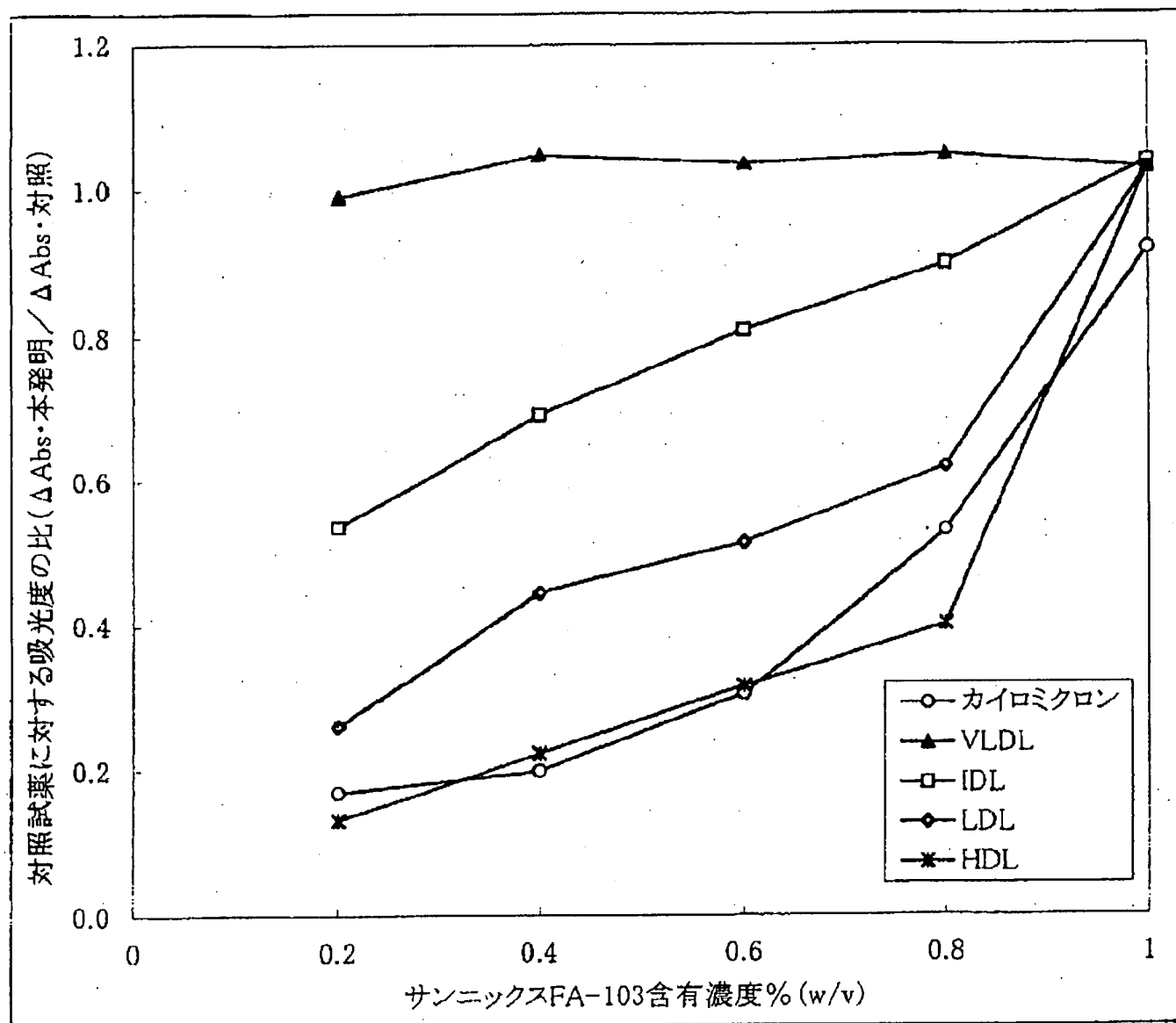
F I G . 1



n-ヘプチル-β-D-チオグルコシド濃度(%)

[Drawing 2]

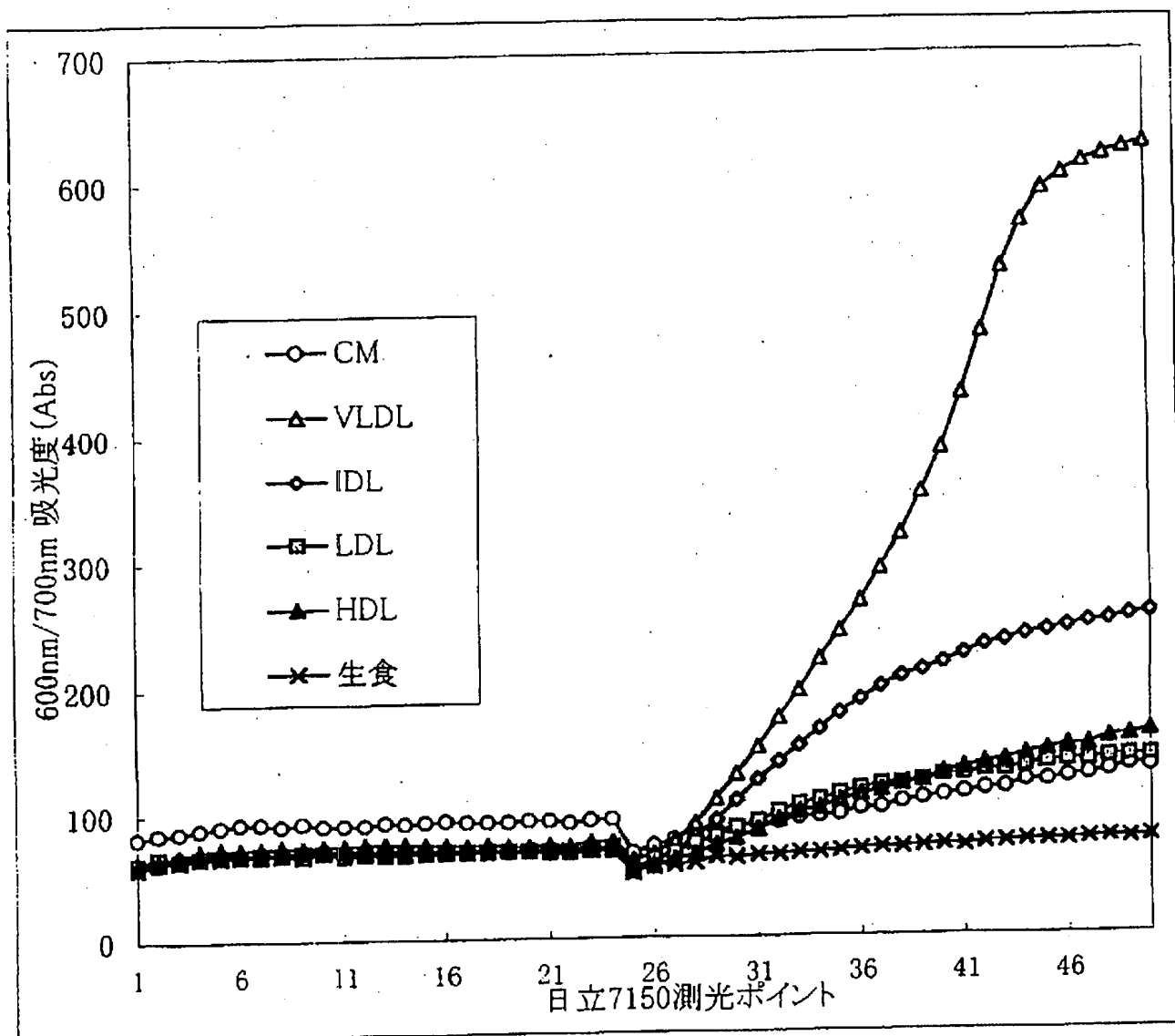
FIG. 2



CM:カイロミクロン
 VLDL:超低比重リポ蛋白
 IDL:中間比重リポ蛋白
 LDL:低比重リポ蛋白
 HDL:高比重リポ蛋白

[Drawing 3]

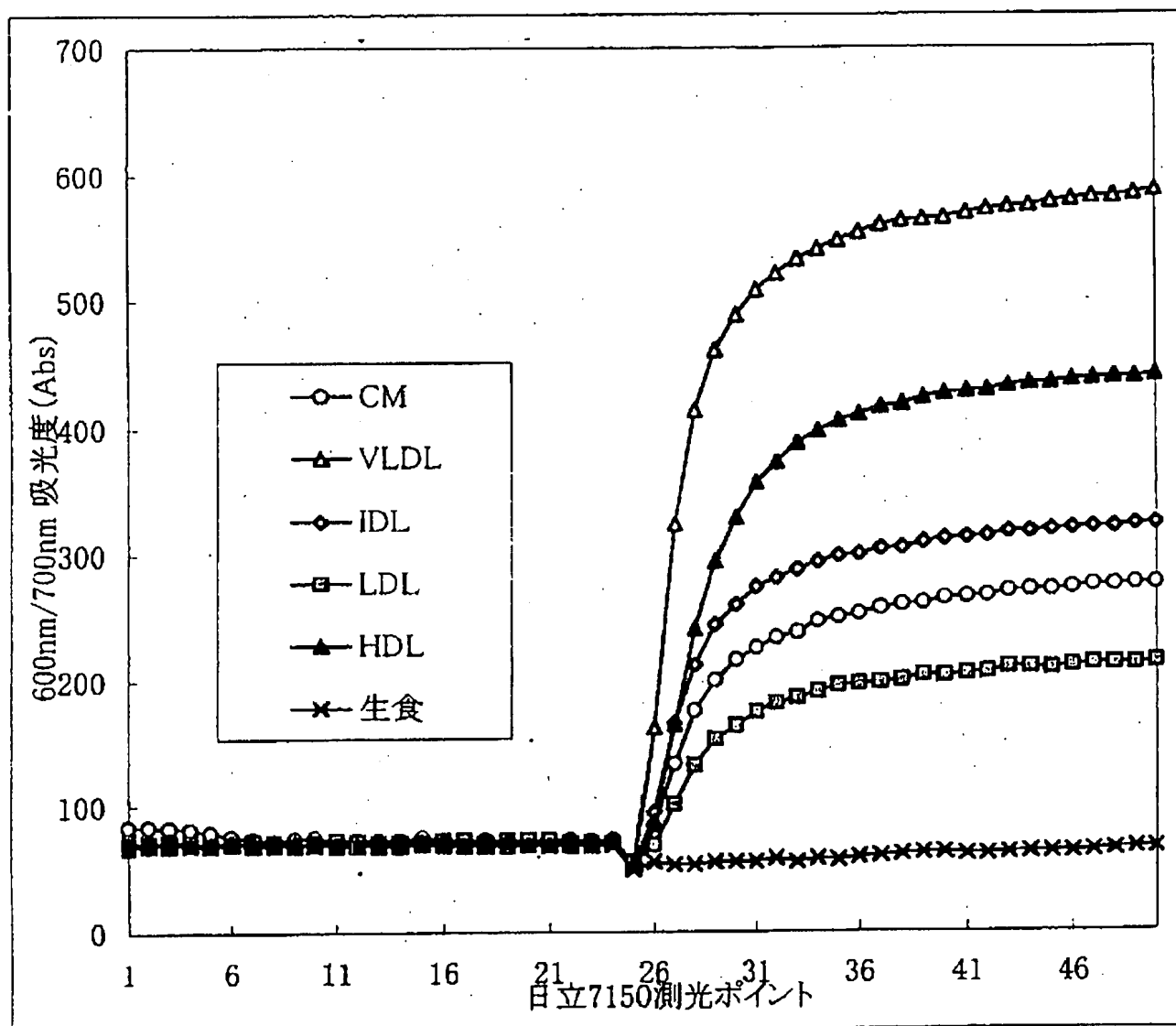
FIG. 3



CM:カイロミクロン
 VLDL:超低比重リポ蛋白
 IDL:中間比重リポ蛋白
 LDL:低比重リポ蛋白
 HDL:高比重リポ蛋白

[Drawing 4]

FIG. 4



CM: カイロミクロン
 VLDL: 超低比重リポ蛋白
 IDL: 中間比重リポ蛋白
 LDL: 低比重リポ蛋白
 HDL: 高比重リポ蛋白

[Translation done.]